Glacial Tree Physiology: Using Stable Isotopes to Reconstruct Plant Responses to Environmental Change Since the Last Glacial Period

BY

Laci M. Gerhart Barley

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Chairperson Dr. Joy K. Ward

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Dr. Sharon Billings

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Dr. Bryan Foster

_____
Dr. Luis González

_____
Dr. Lena Hileman

Date Defended: March 25, 2013

The Dissertation Committee for Laci M. Gerhart Barley
certifies that this is the approved version of the following dissertation:

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Chairperson Dr. Joy K. Ward

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Abstract

Increases in atmospheric [CO_2] (CO_2 concentration) over the last several hundred years have resulted in a current level of just under 400 ppm and represent novel conditions for modern plants relative to their glacial counterparts. Glacial plants experienced consistent oscillations in [CO_2] between 180 and 270 ppm coinciding with glacial-interglacial cycles of the last ~1 million years. Studies of modern plants grown under glacial [CO_2] show severe and consistent negative responses in physiology and biomass; however, detailed analysis of glacial plant material remains limited. Investigation of long-term plant responses to changes in atmospheric CO_2 levels provides important information on glacial plant physiological patterns as well as ecosystem-level processes such as primary productivity and terrestrial carbon storage.

To assess plant responses to low [CO_2] over geologic time scales, preserved glacial wood material was analyzed and compared to modern trees from the same regions. Glacial *Juniperus* specimens spanning the last 50,000 years were obtained from the La Brea tar pits in Los Angeles, CA. Glacial *Agathis* specimens, 50,000+ years old, were obtained from peat bogs in North Island, New Zealand. In both systems, ring width and carbon isotope analysis was performed to compare physiological responses to changes in [CO_2] and environmental factors since the last glacial period. Carbon isotopic signatures were used to calculate c_i/c_a (the ratio of internal CO_2 availability to that of the atmosphere) and c_i. Oxygen isotope analysis was also performed on *Juniperus* to analyze responses to anomalous events, specifically El Niño years.

Both *Juniperus* and *Agathis* showed constant mean c_i/c_a between the last glacial period and modern times. Glacial mean c_i was half the modern c_i levels in both species. These results suggest severe carbon limitations in glacial trees, which could have impacted primary productivity and annual growth patterns. Despite having less than half the available carbon,

glacial *Juniperus* and *Agathis* were able to maintain similar growth patterns to their modern counterparts. We attribute this lack of CO_2 fertilization on tree growth to environmental constraints specific to each region, and constraints resulting from adaptations to 10-14 million years of low CO_2 conditions.

Oxygen isotope analysis was performed on glacial and modern *Juniperus* to reconstruct El Niño impacts in southern California over the last glacial period using a Bayesian model developed on low-elevation southern California *Juniperus*. Under less water-limited growing conditions, modern *Juniperus* from higher elevations do not respond as strongly or as predictably to ENSO-induced changes in temperature and precipitation. This result suggests the same could be true for glacial trees, which could confound proxy-based results in this region. A deeper understanding of the climate-physiology relationship of a species under different environmental conditions is required before a reliable paleo-proxy can be developed.

This research advances our understanding of plant responses to glacial conditions. Carbon isotope analysis provides some of the first direct evidence that glacial plants remained near their lower carbon limit throughout the last glacial period. The ring width analysis shows that operating under limiting carbon conditions did not reduce growth in glacial trees, likely due to environmental constraints on growth, and adaptive and evolutionary constraints to utilizing higher [CO_2] availability. The oxygen isotope analysis indicates altered physiological strategies under less water-limited growing conditions, which impact the strength of plant responses to anomalous climatic events. Collectively, these results have serious implications for understanding of glacial plant function, estimating ecosystem-scale responses such as primary productivity, and developing paleo-proxies for global atmospheric circulation patterns.

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Introduction: Tansley Review of Plant Responses to Low [CO_2] of the Past

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Summary

During the Last Glacial Maximum (LGM; 18,000–20,000 yr ago) and previous glacial periods, atmospheric [CO_2] dropped to 180–190 ppm, which is among the lowest concentrations that occurred during the evolution of land plants. Modern atmospheric CO_2 concentrations ([CO_2]) are more than twice those of the LGM and 45% higher than pre-industrial concentrations. Since CO_2 is the carbon source for photosynthesis, lower carbon availability during glacial periods likely had a major impact on plant productivity and evolution. From the studies highlighted here, it is clear that the influence of low [CO_2] transcends several scales, ranging from physiological effects on individual plants to changes in ecosystem functioning, and may have even influenced the development of early human cultures (via the timing of agriculture). Through low-[CO_2] studies, we have determined a baseline for plant response to minimal [CO_2] that occurred during the evolution of land plants. Moreover, an increased understanding of plant responses to low [CO_2] contributes to our knowledge of how natural global change factors in the past may continue to influence plant responses to future anthropogenic changes. Future work, however, should focus more on the evolutionary responses of plants to changing [CO_2] in order to account for the potentially large effects of genetic change.

I. Introduction

Rising atmospheric [CO_2] (CO_2 concentration) is expected to increase global

temperatures and has been deemed a major threat to climate, economic development, and human health (IPCC, 2007a). It is perhaps ironic to be considering plant responses to low [CO_2] during an era when most research has focused on rising [CO_2]. Here we will emphasize that a strong foundation in understanding of plant responses to low [CO_2] is critical for predicting the implications of rising [CO_2], explaining plant evolutionary patterns over geologic time scales, and estimating past and future levels of net primary productivity (NPP). In addition, low [CO_2] studies provide a baseline for defining plant response to minimum [CO_2] of the geologic past, such that changes in plant functioning in response to rising [CO_2] can be assessed within a broader temporal context.

Atmospheric CO_2 is the main source of carbon for photosynthesis and serves as a fundamental substrate for plant growth. CO_2 assimilation during photosynthesis represents a critical exchange of carbon between the atmosphere and the biosphere within the global carbon cycle (Schlesinger, 1997). CO_2 is unique in that plants assimilate this resource from the atmosphere, whereas other resources such as water and nutrients are acquired from the soil. Furthermore, CO_2 is evenly distributed throughout the Earth's atmosphere, and therefore CO_2 availability to plants is similar across all terrestrial ecosystems (although there can be local gradients with altitude and within forest canopies). This is unlike other plant resources such as light, water, and nutrients that vary across ecosystems, as well as at much smaller spatial scales. Although similar within a time period, [CO_2] has varied throughout geologic time, and during some periods may have been so low as to greatly limit plant growth and reproduction (Ward, 2005). Owing to the inability to substitute "space for time", our knowledge of the evolutionary responses of plants to low [CO_2] is rather limited compared with our understanding of evolutionary responses to other resource limitations, although recent advancements have been

made in this area (and are reviewed here).

Beginning with the rise of vascular land plants through modern times, atmospheric [CO_2] has reached maximum values of 3300-3600 ppm during the early Devonian (Berner, 2006), possibly dropped as low as 150 ppm during the Late Pliocene (Tripati *et al.*, 2009), and consistently ranged between 170 and 190 ppm during glacial maxima of the past million years (Petit *et al*., 1999; EPICA, 2004; Brook, 2005; Fig. 0.1). Since CO_2 is a major substrate for photosynthesis, such extreme changes in the availability of this resource likely had profound effects on plant productivity, community structure, and evolution through time. The Last Glacial Maximum (LGM) that occurred 18,000-20,000 years ago represents a fascinating time when low [CO_2] likely constrained the physiological functioning of C_3 plants (Polley *et al*., 1993a; Dippery *et al*., 1995; Sage & Coleman, 2001; Ward *et al*., 2005; Lewis *et al*., 2010). During that period, [CO_2] dropped to 180-190 ppm (Petit *et al*., 1999; EPICA, 2004), which is among the lowest concentrations predicted to have occurred during the evolution of land plants (Berner, 2003; Berner, 2006; Tripati *et al.*, 2009). Following the LGM, [CO_2] gradually increased to 270 ppm just before to the Industrial Revolution, and has been rising rapidly in recent decades as a result of expanding industrialization (IPCC, 2007b). Currently, [CO_2] is 392 ppm (recorded at Mauna Loa Observatory, Hawaii; http://www.CO_2now.org), a value that may not have occurred since the mid-Miocene, (14-16 million years ago; Tripati *et al.*, 2009). Thus, modern [CO_2] values are more than twice the minimum concentrations that occurred during the LGM and approximately 45% higher than pre-industrial levels. [CO_2] is expected to continue rising in the future, potentially reaching 1000 ppm by 2100, depending on the carbon emissions scenario that actually occurs (see IPCC Working Group I, 2007).

II. The case for low [CO_2] studies

To date, most CO_2 studies have focused on elevated [CO_2] that is predicted to occur within the next 50-100 years as a result of anthropogenic carbon emissions (McLeod & Long, 1999; Ainsworth & Long, 2005; Körner, 2006; Miyagi *et al*., 2007; Springer & Ward, 2007; Lloyd & Farquhar, 2008). The motivation for many of these studies has been to determine the degree to which plants will serve as sinks to offset carbon emissions. Far fewer studies have focused on plant responses to low [CO_2] of the past even though this work is crucial for understanding long-term responses of plants to changing [CO_2] over geologic and evolutionary time scales. From the studies that have been conducted, it is clear that modern C_3 plant genotypes grown at low [CO_2] (180-200 ppm) exhibit severe reductions in photosynthesis, survival, growth, and reproduction, suggesting that low [CO_2] during glacial periods may have induced carbon limitations that would have been highly stressful on C_3 plants (Polley *et al*., 1993, Dippery *et al*., 1995; Sage, 1995; Tissue *et al*., 1995; Sage and Coleman, 2001; Ward and Kelly, 2004; Tonsor and Scheiner, 2007; see Fig. 0.2 for a photo of plants from Dippery *et al*., 1995). In addition, carbon limitations at low [CO_2] may have altered plant tolerance ranges to other stressors such as drought, heat, and herbivory (Sage and Cowling, 1999). Furthermore, most studies focusing on the full range of plant responses to past through future [CO_2] report much greater physiological and growth enhancements in response to increases in [CO_2] below modern concentrations relative to above modern concentrations (Sage and Reid, 1992; Polley *et al*., 1993b; Dippery *et al*., 1995; Ward & Strain, 1997). Thus, plants may have already exhausted much of their potential to respond to rising [CO_2] (unless, for example, major evolutionary changes occur in the future). From these findings, it is clear that assessing the full continuum of plant responses to changes in atmospheric [CO_2] through geologic time is essential for making accurate predictions

regarding the characteristics of both past and future ecosystems.

Studies addressing the effects of low [CO_2] on plants are also fundamental for understanding plant evolution in response to changes in resource availability through time – primarily since changing [CO_2] has been shown to have major implications for plant fitness (Ward *et al*., 2000). Modern plants grown at low [CO_2] (150-200 ppm) exhibit highly compromised survival (Ward & Kelly, 2004) and reproduction (Dippery *et al*., 1995) at conditions that occurred only 18,000-20,000 years ago. Such findings beg the question of how glacial plants survived during low [CO_2] periods, especially considering the lack of evidence for plant extinctions during these times. Furthermore, past work has demonstrated that low [CO_2] has the potential to act as a strong selective agent on plants, and therefore, evolutionary responses may have ameliorated some of the negative effects of low [CO_2] in the past (Ward *et al*., 2000). However, the full suite of mechanisms accounting for these adaptive responses is currently unknown, as well as how adaptive processes may have been influenced by other interactions climate (for a discussion of possibilities, see Sage, 1994; Sage & Cowling, 1999; Ward *et al*., 2000; Beerling, 2005). Furthermore, it is also important to consider that any genetic changes that occurred in the recent geologic past as a result of low [CO_2] may continue to affect the responses of plants to rising [CO_2] throughout the next century (Strain, 1991; Sage & Cowing, 1999).

Overall, low [CO_2] studies are critical for understanding plant responses to past environments when carbon resources were most limiting, evaluating physiological and growth constraints for response to rising [CO_2], determining the full continuum of plant responses to changes in [CO_2] over evolutionary time scales, assessing the impacts of low [CO_2] on plant community composition and ecosystem functioning, and understanding the influence that low

[CO_2] may have had on early human cultures via influences on the development of agriculture. Moreover, studying plant responses to low [CO_2] provides information about past ecosystem functioning, such as estimates of glacial NPP (Prentice & Harrison, 2009), as well as insights into the availability of food resources for animals (Coltrain *et al*., 2004) and early humans (Sage, 1995; Richerson *et al*., 2001).

In this review, we mainly focus on the effects of low [CO_2] that occurred during glacial periods, with an emphasis on plants possessing the C_3 photosynthetic pathway (85-90% of all species), since they tend to be most responsive to low [CO_2] (Ehleringer *et al*., 1991; Ehleringer *et al*., 1997). For our purposes, "low" or "glacial" CO_2 corresponds to values of 170-200 ppm, "pre-industrial" values correspond to 250-300 ppm, "modern" values range between 350-400 ppm, and "elevated" values correspond to [CO_2] at or greater than 500 ppm. Although numerous environmental factors have changed between glacial and interglacial periods (e.g. precipitation, temperature), few studies have focused on the interactive effects of low [CO_2] with other variables. We incorporate interactive effects with low [CO_2] when possible, recognizing that our understanding in this area is limited.

We emphasize that the majority of low [CO_2] studies involve the use of modern plants grown at simulated conditions of the past. Such studies allow us to characterize the effects of limiting [CO_2] on physiological, growth, and reproductive processes. Unfortunately, however, because these studies generally report on only a single generation of growth at low [CO_2], they limit the scope of our understanding of evolutionary processes. Therefore, we also discuss the results of studies that incorporate fossil material from glacial plants that were more fully adapted to low [CO_2] as well as studies focusing on the evolutionary responses of plants to low [CO_2] over multiple generations of artificial selection. We also fit low [CO_2] research into the larger

context of global change studies, discuss technologies for reducing [CO_2] during experimentation, and provide recommendations for future research directions in the field.

III. Experimental approaches for reducing [CO_2]

Methods for elevating [CO_2] above ambient concentrations are often achieved via external sources of CO_2 and have been applied to growth chambers, open-top chambers, and Free Air CO_2 Enrichment (FACE) sites, providing multiple scales for elevated [CO_2] research. Reducing [CO_2] below the modern value is much more difficult, since CO_2 gas must be scrubbed from the atmosphere rather than added to it. This presents a major challenge when there is a large volume of air that must be scrubbed of CO_2 in a controlled and rapid manner. Several approaches have been developed to address this issue, and the benefits and limitations of each are discussed in the following.

The earliest methods for reducing [CO_2] took advantage of the finding that C_4 plants can outcompete C_3 plants under limiting [CO_2]. In order to reduce [CO_2], researchers enclosed C_4 plants side-by-side with experimental C_3 plants in small chambers, effectively lowering [CO_2] below the C_3 compensation point ([CO_2] where carbon gain through photosynthesis equals carbon loss from respiration; e.g. Sharma *et al*., 1978; Fig. 0.3a). This approach did not allow for tight control of [CO_2], and resulted in rapid onset of carbon starvation in plants, but was a clever way to lower [CO_2] before the introduction of more sophisticated products and methods.

In the mid 1990s, a later phase of technology was introduced at the Grassland, Soil, and Water Research Laboratory (USDA-Agricultural Research Service) that involves an outdoor tunnel system, whereby plants are grown across a continuum of modern to low [CO_2] (see Mayeux *et al*., 1993; Fig. 0.3b,c). During the day, air of known [CO_2] is pumped into one end of

the tunnel where plants experience relatively high [CO_2]. Plants near the end of the tunnel, on the other hand, experience low [CO_2] (similar to glacial values) as a result of photosynthetic removal of CO_2 from air as it moves progressively through the tunnel. At night, airflow is reversed while plants are solely respiring, and this serves to equalize [CO_2] throughout the whole tunnel. The desired [CO_2] is maintained by varying the rate at which air flows through the chamber in response to changes in photosynthetic and respiration rates. A major strength of this system is that these chambers are housed outdoors and are exposed to full, natural lighting conditions with tight temperature control. In addition, plant data acquired from these experiments can be analyzed across a [CO_2] gradient, as opposed to using discrete CO_2 treatments. A disadvantage, however, is that the [CO_2] gradient cannot be maintained during dark hours, and sample size within a specific, targeted [CO_2] may be limited.

A different approach to lowering [CO_2] is through the use of controlled growth chambers, which can range in size from small reach-in varieties (e.g. Ward *et al*., 2000; Mohan *et al*., 2004, Sage & Reid, 1992; Fig. 0.3e) to large walk-in chambers commonly housed in experimental phytotrons (e.g. Ward *et al*., 2008). In these chambers, plants can be grown with a variety of options, such as computer-controlled temperature, humidity, light and CO_2 conditions. Most commonly, low CO_2 treatments are obtained by scrubbing CO_2 from the atmosphere, either by forcing air through "scrub boxes" containing soda lime (contents may include calcium hydroxide, sodium hydroxide, and/or potassium hydroxide depending on the manufacturer; Fig. 1.3d) or by passing compressed air across soda lime filters. Even in these small and highly controlled systems, the maintenance of [CO_2] can be inherently difficult. We have found that the best results occur when the scrubbing of CO_2 within scrub boxes (Fig. 0.3d) is coupled with CO_2 additions from an external tank with a computer-generated switch, allowing for more constant

[CO_2] control within the chamber (i.e. competing additions and scrubbing of CO_2 gas tend to stabilize [CO_2]; JK Ward, unpublished). In addition, other researchers have utilized a system where CO_2-free air is emitted into enclosed chambers in order to lower [CO_2]. These units at the University of Florida (Gainesville, Florida, USA) were made of glass and were housed outdoors, allowing for natural, full sun conditions (Baker *et al*., 1990; Allen *et al*., 1998). Overall, the tight control offered by the experimental systems described earlier is accompanied by the loss of realistic field conditions, and therefore these approaches may be less informative for strict ecological questions. These systems, however, can be highly useful for uncovering basic genetic, physiological, and growth mechanisms that may be driving individual plant responses to low [CO_2].

In order to advance low [CO_2] research, it is hoped that future approaches will combine technologies for reducing [CO_2], maintenance of environmental control, as well as employing conditions that more closely simulate natural, field conditions. Such plans are currently under consideration and are being discussed among the scientific community at large.

IV. Early low [CO_2] studies

The earliest studies focusing on plant responses to low [CO_2] began in the early 1960s and were prompted by the development of new technologies for measuring plant gas exchange. These studies focused on determining the CO_2 compensation point for plants, as well as comparing respiration and photosynthetic rates among species and genotypes. For example, Moss (1962) grew a variety of economically important crops (corn, sugar cane, orchard grass, tobacco, geranium, tomato, and Norway maple) in a closed system and allowed plants to draw down CO_2 over time. The author found that corn and sugar cane (now known to be C_4 plants)

could draw down [CO_2] below 10 ppm, whereas the other species (now known to be C_3 plants) could only draw down [CO_2] between 60 and 145 ppm. Interestingly, this low [CO_2] study distinguished plants with the C_4 versus C_3 photosynthetic pathways several years before the formal discovery of C_4 photosynthesis. At the time, the author was unable to provide a specific mechanism to account for these differences, but realized he was working with two unique physiological systems. It was later shown that C_4 plants have a CO_2 compensation point that is close to 0 ppm, whereas that of C_3 plants is in the vicinity of 50-60 ppm between 25 and 30° C.

During the early 1960s, Billings *et al*. (1961) also dealt with plant responses to low [CO_2], but from a more ecological perspective. The authors compared gas exchange rates of two populations of *Oxyria digyna* – one from high altitude (Logan Pass, Montana, USA) and the other from sea level (mouth of the Pitmegea River, Alaska, USA). Seeds from these populations were grown under common conditions and photosynthetic rates were measured across a wide range of [CO_2]. The authors found that photosynthetic rates were higher and CO_2 compensation points were lower in offspring that originated from alpine populations, and suggested that this may reflect adaptations to lower CO_2 partial pressure at high altitudes. The authors also concluded that low CO_2 partial pressure, and not temperature, limited the upward distribution of the *Oxyria* populations in this case. A subset of more recent studies have supported these findings, while others have pointed out that there may be alternative explanations for these results. Similar to the findings of Billings *et al.* (1961), Körner & Diemer (1994) showed that species of the genera *Ranunculus* and *Geum* exhibited higher carbon gain when grown at elevated [CO_2] compared with lowland species of the same genera. In addition, Ward & Strain (1997) found that *Arabidopsis* genotypes from high elevations produced greater seed numbers (indicating higher fitness) when grown at low [CO_2] (200 ppm) compared with genotypes from

lower elevations (where CO_2 partial pressure was higher). Other studies, however, have noted additional factors with increasing altitude that would serve to enhance photosynthesis, thus overcoming some, if not all, of the negative effects of low CO_2 partial pressure (Gale, 1972; Sage & Reid, 1992; Terashima *et al.*, 1995). These include increased diffusivity of CO_2 at higher elevation that facilitates the movement of CO_2 from air to intercellular spaces, as well as reductions in O_2 partial pressure with elevation (proportionally similar to CO_2) that would reduce photorespiration. These factors can potentially offset the drop in CO_2 partial pressure up to 2500 m, after which stromal [CO_2] may be reduced by 20% (although here low temperatures may begin to limit carbon gain over CO_2; Sage & Reid, 1992). Taken together, these considerations suggest that high-altitude plants may not serve as a viable model for understanding adaptive responses of plants to global reductions in [CO_2] of the past (Terashima *et al.*, 1995).

A new era of low [CO_2] studies began during the late 1960s, with the objective of identifying plants with high photosynthetic efficiency and low photorespiration rates, with the overall goal of improving crop productivity. This research occurred in conjunction with the Green Revolution that emphasized initiatives for increasing food production. In several studies (Cannel *et al*., 1969; Nelson *et al*., 1975; Sharma *et al*., 1979), both crop and model plants were grown at extremely low [CO_2] that was near or below the CO_2 compensation point of C_3 plants (achieved by growing newly identified C_4 plants alongside experimental C_3 plants). Subsequently, genotypes were screened for survival at these extremely limiting carbon conditions with the assumption that surviving genotypes would exhibit superior photosynthetic efficiency. While mostly unsuccessful with respect to the original goal, these studies provided valuable insights into the degree of physiological stress induced by low [CO_2]. For example,

Cannell *et al*. (1969) screened 2,458 genotypes of soybean (*Glycine max*, C_3) and found that none could survive at low [CO_2] near the compensation point (ranging between 10-50 ppm CO_2). A decade later, Sharma *et al*. (1979) conducted studies with 33 *Arabidopsis thaliana* (C_3) genotypes grown below the CO_2 compensation point. They found considerable variation, whereby some genotypes survived only one week at these conditions, whereas others could survive longer than two weeks (relatively large variation for such a short-lived species). They also noted that the crossing of parents with extreme differences in survival time near the CO_2 compensation point resulted in a highly variable F_2 population (with some genotypes being superior to both parents), suggesting that there is considerable natural genetic variation for survival at limiting [CO_2]. In more recent advances (described in Delgado *et al*., 1994; Medrano *et al*., 1995), haploid tobacco (*Nicotiana tabacum*, C_3) lines derived from mutagen-treated anthers were selected for survival near the CO_2 compensation point (60-70 ppm). Approximately 5% of the lines survived, and of those, plants had greater total leaf area, maintained higher photosynthetic rates specifically in mature leaves and older leaves, and had lower respiration rates (on a leaf mass basis, but not an area basis) relative to parental plants. This study, however, did not find alterations in photorespiration rates and properties of Rubisco in selected plants, as originally hoped.

In the 1980's, the publication of ice core data characterized the CO_2 composition of the atmosphere during the late Pleistocene, Holocene, and pre-industrial periods. Consequently, studies began focusing on the effects of low [CO_2] on plants from a geological and historical perspective. One of the first of these studies was conducted by Overdieck *et al*. (1988), who found that C_3 herbaceous annuals (*Vigna unguiculata* L., cowpea; *Abelmoschus esculentus* (L.) Moench, okra; *Raphanus sativus* L., radish) exhibited an average 8% reduction in growth at the

pre-industrial value of 270 ppm CO_2 relative to the modern value that was 350 ppm at the time. These results suggested that anthropogenic increases in [CO_2] over contemporary time scales may have already modified plant functioning and productivity within modern ecosystems. Using *Lyonia mariana*, Overdieck was among the first to show that plants grown at pre-industrial [CO_2] exhibited greater water loss as a result of higher stomatal conductance (g) (by as much as 16%) compared with plants grown at modern [CO_2] (Overdieck, 1989). More recent studies have generally confirmed this result with instantaneous gas exchange measurements, but few studies have allowed for full physiological acclimation to pre-industrial [CO_2] over a full generation as in the Overdieck study. In a more recent study, Dippery *et al*. (1995) found that the biomass production of *Abutilon theophrasti* was reduced by 24% when grown at pre-industrial [CO_2] (270 ppm) versus modern value (350 ppm) (see Fig. 0.2 for a photograph), although specific effects of [CO_2] on physiology and other leaf properties could not be identified between these treatments (Tissue *et al*., 1995).

In summary, these early studies laid the groundwork for more recent studies by showing that plants with different photosynthetic pathways exhibit differential responses to low [CO_2], modern C_3 plants can become highly stressed when grown at low [CO_2], and plants exhibit genetic variation in response to low [CO_2] (whether natural or induced) that is often explained by shifts in biomass allocation or developmental timing rather than through direct alterations in photosynthesis/photorespiration rates or photosynthetic enzyme characteristics.

In the remaining sections, we describe the results of more recent low [CO_2] studies that investigate a variety of scales ranging from physiological to ecosystem-scale processes. We also address the potential for plant evolutionary responses to low [CO_2] by reviewing studies that focus on plant fossils that occurred during glacial periods, as well as modern plants that were

selected for high fitness over multiple generations at low [CO_2]. We also discuss the current status of a hypothesis by Sage (1995), suggesting that low [CO_2] may have influenced the timing of agriculture among early humans. Although we are unable to describe all low [CO_2] studies in detail, we highlight a wide range of work that reflects our current understanding of plant responses to low [CO_2].

V. Low [CO_2] effects on the individual plant

1. Physiological responses

In C_3 plants, low [CO_2] affects net photosynthetic rates by reducing the rate of carboxylation of rubisco resulting from substrate limitations and through higher photorespiration rates. Photorespiration is increased at low [CO_2] because both CO_2 and O_2 compete for the same active site of rubisco. A reduction in [CO_2]/[O_2] enhances oxygenation, resulting in carbon loss to the plant. Note that unlike [CO_2], [O_2] has remained unchanged in the atmosphere for at least the last several million years (Berner *et al*., 2007), and thus [O_2] changes are not relevant to the time periods covered in this review.

Studies that measure plant responses to low [CO_2] most commonly involve growing plants at modern [CO_2], followed by instantaneous gas exchange measurements on a small portion of leaf area across a large range of [CO_2] (typically ranging between 0 and 1000 ppm). Referred to as A-c_i curves (photosynthetic assimilation versus leaf inter-cellular [CO_2]), these measurements encompass low [CO_2] that was characteristic of glacial periods (Fig. 0.4). A-c_i curves generally show steep linear increases in net photosynthetic rates (A) between c_i values of 0 and 250 ppm CO_2, with continued positive slopes through c_i values as high as 1000 ppm (Sage & Coleman, 2001). These curves indicate that reductions in [CO_2] during glacial periods

produced rubisco-limited photosynthetic rates (Sage & Coleman, 2001). A survey of A-c_i curves from a variety of C_3 species indicate that net photosynthetic rates are reduced by 40-60% at low (180 ppm) versus modern (350-380 ppm) [CO_2] (c_a values), with approximately 30% of this reduction being induced by higher photorespiration rates when temperatures are optimal (Sage & Cowling, 1999).

A-c_i curves are highly informative for understanding the biochemical properties of rubisco in response to changing [CO_2]. Because this enzyme has been highly conserved across evolutionary time scales (Griffiths, 2006), such measurements may have the potential to be extrapolated to glacial plants. It should be noted, however, that the extent of physiological acclimation at each [CO_2] measurement is short-term in nature, and thus full acclimation responses are rarely assessed with A-c_i curves. To further highlight this point, changes in A-c_i curves have been observed in C_3 plants grown at modern [CO_2] and then transferred to low [CO_2] (200 ppm) for several weeks, and the direction of change was generally unpredictable (Sage, 1994; also see Overdieck, 1989; Sage & Reid, 1992). In addition, such measurements do not provide insights into whole-plant responses to low [CO_2] over the full life cycle, such as modifications in both relative and total leaf area that can influence whole-plant carbon uptake. Another point worth noting is that the A-c_i curves of plants that are fully adapted to low [CO_2] (over many generations of genetic change) may potentially deviate from that of modern plants, thus limiting the potential for physiological extrapolation to the past. Because we know essentially nothing about this issue, future work should focus on the extent to which modern physiological responses can be extrapolated to the past, with the inclusion of full acclamatory and adaptive processes.

In addition to having direct effects on photosynthetic rates and carbon gain, changes in [CO_2] can also have large effects on g that can include transpiration rates and water use efficiency (WUE; carbon assimilation/stomatal conductance). In the vast majority of C_3 plants, g increases with reductions in [CO_2] (by 35-50%, depending on the species) between modern (350-380 ppm) and glacial values (180-200 ppm). This response is observed during short-term gas exchange measurements (Lloyd *et al*., 1992; Flexas *et al*., 2007), as well as long-term exposure to low [CO_2] over a full generation (Polley *et al*., 1993b; Ward *et al*., 1999; Tonsor & Scheiner, 2007). Higher g serves to enhance CO_2 uptake by reducing the diffusional resistance of CO_2 into the leaf interior, but with the cost of higher water loss. Furthermore, Polley *et al*. (2002) found that higher g within a C_3/C_4 grass assemblage grown at low [CO_2] translated into greater depletion of soil water than seen in similar assemblages grown at modern [CO_2] (Polley *et al*., 2002), suggesting that stomatal responses to low [CO_2] can have implications for water availability at the ecosystem level.

Using carbon isotopes ratios, Polley *et al*. (1995) estimated WUE in a variety of C_3 plants (*Triticum aestivum, Bromus tectorum, Prosopis glandulosa*) and found reduced values at low versus modern [CO_2]. Interestingly, reductions in WUE were directly proportional to reductions in [CO_2] in all species, suggesting that the ability to scale this response may be relatively straightforward. In the same study, nitrogen use efficiency (NUE; biomass produced/plant N) decreased in *B. tectorum* and *P. glandulosa* at low [CO_2], but this response was not as pronounced or consistent as changes in WUE, suggesting that low [CO_2] may have increased the requirements for water to a greater extent than N in C_3 species.

2. Biomass production

In order to determine how physiological responses to low [CO_2] manifest themselves at the whole-plant level, plants must be grown for a full generation at low [CO_2]. Such studies have shown that the average biomass production of modern C_3 plants is reduced by approximately 50% when grown at low (180-220 ppm CO_2) versus modern [CO_2] (350-380 ppm), when other conditions are optimal (Sage & Coleman, 2001; Fig. 0.5). There is, however, variation in this response among C_3 species (Fig. 0.5), as well as within C_3 species, whereby reductions in biomass may vary by 40-70% among genotypes (Ward & Strain, 1997; Hovenden & Schimanski, 2000; Mohan *et al*., 2004). In addition, as [CO_2] declines to 150 ppm, biomass production may be reduced by as much as 92%, as was observed in *A. theophrasti* (Dippery *et al*., 1995; Figs. 0.2 & 0.5).

It is also generally found that the same absolute change in [CO_2] *below* the modern value has a much greater effect on biomass production (and A) than the same absolute change in [CO_2] *above* the modern value (Allen *et al*., 1991; Polley *et al*., 1992; Dippery *et al*., 1995; Tissue *et al*., 1995; Ward & Strain, 1997; Ward, 2005; Fig. 0.5). Thus, even small changes in [CO_2] during glacial periods may have had large effects on plant productivity. It is possible, however, that modern plant responses to past [CO_2] may be accentuated relative to glacial plants that were more fully adapted to low [CO_2]. Moreover, the lack of evidence for plant extinctions during glacial periods indicates the likelihood that plants underwent adaptive changes in response to low [CO_2] that may have altered biomass production, and these responses may no longer be evident in some modern genotypes (Ward *et al*., 2000; Ward & Kelly, 2004; Tonsor & Scheiner, 2007).

When comparing biomass production and net photosynthetic rates (A) of C_3 plants, there is often a stronger correlation between these measurements at low [CO_2] compared than at

modern or elevated [CO_2] (Tissue *et al*., 1995; Sage & Coleman, 2001; but also see Cowling & Sage, 1998 where this correlation becomes decoupled at very high temperature). This finding indicates that photosynthate is more directly converted into biomass at low [CO_2], and this is often accompanied by lower starch and sugar accumulation within leaves (Sage, 1995; Allen *et al*., 1998). Furthermore, lower photosynthetic rates at low [CO_2] often persist throughout a full generation, contributing to sustained reductions in biomass production. This is the case even when plants undergo acclimation response to low [CO_2] such as higher concentrations of Calvin-Benson cycle enzymes (mainly rubisco; Tissue *et al*., 1995; but also see Gesch *et al*., 2000 for an example of partial recovery), higher g (Polley *et al*., 1993b; Ward *et al*., 1999), increased activity of carbonic anhydrase that facilitates CO_2 diffusion into the chloroplasts (Coleman, 2000), and greater leaf area ratio (leaf area/total plant mass, LAR) that may enhance whole-plant carbon uptake (Dippery *et al*., 1995).

3. Biomass partitioning

It has been hypothesized that plants grown at low [CO_2] would partition a higher proportion of biomass to above-ground than to below-ground structures; this response would serve to increase LAR and would enhance overall investment in carbon assimilation under limiting [CO_2] (Sage & Coleman, 2001). A variety of studies have found support for this idea, including Dippery *et al*. (1995) who showed that *A. theophrasti* partitioned a higher proportion of biomass to shoots relative to roots at 150 than at 350 ppm CO_2 (root:shoot mass = 0.17 vs. 0.34, respectively); this finding was additionally confirmed by allometric analysis that accounted for ontogenetic shifts (JK Ward, unpublished). Surprisingly, this response ended up being counter-productive, since reduced investment in root production resulted in reduced N uptake

and lower rubisco production, further compounding the negative effects of low [CO_2] on carbon uptake (Tissue *et al*., 1995). Interestingly, Cowling & Sage (1998) observed that *Phaseolus vulgaris* also had reduced partitioning of biomass to roots at low [CO_2], but this response was associated with increased stem biomass as opposed to leaf biomass. Such a finding may be associated with direct effects of [CO_2] on biomass partitioning, or may be the result of indirect effects of shifting plant ontogeny in response to differences in [CO_2]. With respect to contemporary time scales, neither Dippery *et al*. (1995) nor Bunce (2001) found differences in LAR in *A. theophrasti* and other C_3 annuals grown at pre-industrial (270 ppm) versus modern (350-370 ppm) [CO_2]. Also, Ghannoum *et al.* (2010) found no differences in root allocation in two *Eucalyptus* species grown at pre-industrial (290 ppm) and current (400 ppm) [CO_2] at ambient temperature. These findings suggest that increases in [CO_2] over the past hundred years may not have had a large influence on plant biomass partitioning (even though absolute biomass is often affected).

4. Developmental timing

Elevated [CO_2] is known to affect plant developmental timing and such responses have been shown to influence plant fitness (Ward & Kelly, 2004; Springer & Ward, 2007; Springer *et al*., 2008). Unfortunately, very little is known about the effects of low [CO_2] on the developmental timing of C_3 plants. Sage & Coleman (2001) hypothesized that increasing carbon storage within roots before reproduction would enhance fitness at low [CO_2] (assuming there was sufficient time remaining in the growing season to reproduce), and this could be achieved by delaying developmental milestones such as flowering. In support of this idea, Ward & Strain (1997) found that field-collected genotypes of *A. thaliana* required on average 9 days more to

initiate flowering when grown at 200 versus 350 ppm CO_2. This represented a proportionally large shift in developmental timing considering that this species only has a 40-60 day life cycle. Unlike this finding, Polley *et al*. (1993b) did not find modifications in developmental timing in two cultivars of wheat grown from 200 to 350 ppm CO_2. These studies suggest that at this point, there is no predictable response pattern to describe the effects of low [CO_2] on the developmental timing of C_3 annuals and indicate that additional work is needed in this area. Furthermore, little is known about the effects of low [CO_2] on the developmental timing of perennials. It is predicted that they would have an advantage over annuals at low [CO_2] in that reproduction could be delayed until adequate resources were acquired for successful reproduction (Cowling, 2001; Ward, 2005).

5. Reproduction and survival (fitness components)

Of the few studies measuring the survival and reproduction of C_3 plants grown at low [CO_2], all have reported large effects on these measurements. Dippery *et al*. (1995) found the most extreme response, where low [CO_2] (150 ppm) prevented reproduction in the modern C_3 annual, *A. theophrasti,* as a result of the abortion of all flower buds, which drove the fitness response to zero. This finding suggested that 150 ppm CO_2 may be near the threshold for successful completion of the life cycle in some C_3 species. Campell *et al.* (2005) found that tobacco was able to successfully reproduce at both 100 and 150 ppm [CO_2], although after a very large amount of time (16 weeks), and the germination percentage of offspring was compromised at 100 versus 150 ppm. Both the Dippery *et al*. (1995) and Campbell *et al.* (2005) studies provide a strong reminder that the whole-plant CO_2 compensation point allowing for full completion of the plant life cycle, may be substantially higher than the leaf CO_2 compensation point. In

addition, much more time may be required for successful reproduction at low [CO_2] since sufficient carbon must be accumulated and stored in order for reproduction to be successful. Such considerations must be kept in mind when predicting the full effects of low [CO_2] on plant reproduction and fitness.

Tonsor & Scheiner (2007) investigated the effects of CO_2 availability (ranging between 250 and 710 ppm) on patterns of trait integration among 35 genotypes of *A. thaliana*. They found that at low [CO_2], whole-plant carbon assimilation was the main determinant of reproductive mass, although this relationship was not maintained at higher [CO_2]. This study echoes the findings with A and biomass production (see section V.2.), but now additionally shows that A and fitness can also be closely correlated at low [CO_2]. In addition, Ward & Kelly (2004) also worked with *A. thaliana* and found that six field-collected genotypes exhibited reduced survival ranging between 20 and 49% and reduced seed production ranging between 38 and 81% when grown at 200 ppm versus 350 ppm CO_2. As a result, reductions in estimated fitness (percentage survival X total seed production) ranged between 59 and 87% among the genotypes, indicating substantial genetic variation for low [CO_2] response (significant CO_2 X genotype interaction; Fig. 0.6). Furthermore, the reductions in reproductive output among the *Arabidopsis* genotypes were a result of overall reduced plant size, as opposed to changes in the partitioning of biomass to reproduction (Ward & Kelly, 2004). Taken together, these studies, as well as others (also see Mohan *et al*., 2004 with maple) indicate that there is ample genetic variation to account for evolutionary responses to occur at low [CO_2].

In a different type of study using ^{13}C as a label, Lehmeier *et al*. (2005) found that low [CO_2] (200 ppm) did not alter the mobilization rate of seed-derived carbon within newly developing sunflower seedlings; such a response may have influenced survival (not measured in

this study), since rapid movement of carbon reserves would be essential for maintaining a positive carbon balance in seedlings growing at low [CO_2]. In addition, seed size may play a major role in determining survival at low [CO_2], since greater carbon reserves would enhance the production of leaf area within seedlings, reducing the chances of destructive negative carbon budgets during rapid and early growth stages (Ward & Kelly, 2004; Ward, 2005; also see Metz *et al.* 2010 for experimental and theoretical considerations of seed size and survival). Likewise, the small seed size of *A. thaliana* may have contributed to the high mortality rates that were observed at low [CO_2] in the Ward & Kelly (2004) study discussed earlier (Fig. 0.6).

VI. Low [CO_2] and plant evolution

In past work, researchers have speculated about possible evolutionary responses of plants to low [CO_2] during different geologic time periods (Beerling, 2005). For instance, Beerling *et al.* (2001) pointed out an association between decreasing [CO_2] during the late Devonian and the evolution of megaphyll leaves which had higher stomatal densities, higher transpiration rates, and greater capacity for cooling (based on modeling) compared with more primitive leaves. Furthermore, McElwain *et al.* (2005) suggested linkages between declining [CO_2] during the Cretaceous and an increase in the relative abundance and radiation of the angiosperms.

The rise of C_4 photosynthesis is also a major evolutionary event, that has been attributed to low [CO_2] (initially by Ehleringer *et al.* 1991; also in Ehleringer *et al.* 1997; Cerling *et al.* 1998; reviewed by Sage, 2004). Early work suggested that [CO_2] decline during the late Miocene contributed to the evolution of C_4 species since these plants can concentrate CO_2 in bundle sheath cells even when atmospheric [CO_2] is low. More recent work, however, has postulated that C_4 photosynthesis originated much earlier, most likely during the Oligocene (20 million

years earlier) and this idea has yielded phylogenetic support (Sage, 2004). Similar to the initial idea, however, C_4 evolution has still been linked with reductions in [CO_2], although much earlier in time, which became possible as the geologic record of atmospheric [CO_2] improved.

In more recent geologic periods, it is now recognized that there was a major expansion of C_4-dominated grasslands during the late Miocene-early Pliocene that would have resulted in dramatic shifts in plant productivity and ecosystem functioning (Sage, 2004; Tipple & Pagani, 2007; Edwards *et al.*, 2010). This transition occurred during an abrupt [CO_2] decline as recently shown by Tripati *et al.* (2009) using boron:calcium ratios in foraminifera. Their results showed that atmospheric [CO_2] dropped as low as 200-300 ppm between 5 and 10 million years ago, suggesting a likely role for low [CO_2] in this C_4 expansion. However, Tipple & Pagani (2007), and more recently Edwards *et al.* (2010) noted other factors that may have played a role in C_4 expansion, including aridity, a more seasonal climate, fire disturbance, and monsoon. These papers emphasized that this C_4 expansion should be evaluated on a more regional scale, and with attention to multiple C_4 adaptations (in addition to low [CO_2] tolerance), since C_4 expansion does not appear to be globally synchronous. In more recent geologic time, there is strong evidence that C_4 plants continued to expand their range in response to low [CO_2] during glacial periods of the past million years, particularly in the tropics where warmer temperatures would have additionally favored C_4 photosynthesis (see section VIII. For more details). In addition, this has also been deemed a period of recent C_4 evolution, mainly through the rise of a variety of C_4 dicot lineages (Ehleringer *et al.*, 1997; Sage, 2004). Even with this recent geologic expansion of C_4 species, C_3 plants still persisted in many ecosystems during glacial periods (e.g. Coltrain *et al.*, 2004, southern California), and it is not yet fully understood how they functioned and adapted to low [CO_2] during that time. It is clear, however, that modern C_3 plants are products of an

ancestry that had undergone at least 2 million years of glacial-inter-glacial cycles, with corresponding changes in climate and [CO_2] (Ward & Kelly, 2004). Thus, glacial plants must have had the genetic capacity to adapt to changing [CO_2], and mechanisms accounting for this process may still be detectable in some modern species/genotypes. Furthermore, a better understanding of these responses will provide important insights into the capacity for plants to evolve in response to rising [CO_2] in the future, which is a critical issue at this juncture.

While studies conducted for an entire generation at low [CO_2] provide critical information on whole-plant responses with full acclimation (Section V), these studies do not incorporate adaptive changes at low [CO_2] that occur over multiple generations with genetic change. The process of understanding plant evolution at low [CO_2] has been addressed through two main approaches. In the first, modern plants are selected at low [CO_2] (180-200 ppm) for high growth or reproduction, allowing for an understanding of how genetic change may drive adaptive processes in living plants. These studies are conducted over multiple generations and differ from the screening methods described in earlier studies where genotypes were selected for survival during only one generation of exposure to extremely low [CO_2] (e.g. Sharma *et al.* 1979). A second approach involves studying fossilized plant material that actually occurred during glacial periods; such fossils are highly useful for determining evolutionary responses to low [CO_2] since these plants had thousands of years to adapt to these conditions over multiple generations. A relatively large number of plant specimens dating to the LGM have been naturally preserved in tar pits, bogs, and caves, and these may serve as critical resources for further addressing this issue (Wells & Jorgensen, 1964; Stock & Harris, 2001). Unfortunately, however, measurements on these fossils are generally restricted to stable isotopes, morphology, DNA sequencing (in some cases), and modeling approaches because they are non-living. By studying

these specimens, along with modern plants that have been adapted to low [CO_2] over multiple generations, we may better understand how plants functioned across a wider range of [CO_2], and we may be able to pinpoint the mechanisms that enabled C_3 plants to survive during the most limiting [CO_2] periods of the past.

To investigate evolutionary processes, Ward *et al*. (2000) conducted a selection experiment with *A. thaliana* (derived from out-crossings) for high seed number over five generations at low [CO_2] (200 ppm; control plants were also grown side-by-side with selected plants, but were *randomly* selected). At the fifth and final generation of selection, plants from selected populations produced 25% more seeds on average (with no changes in quality) than non-selected control plants when both were grown at low [CO_2] (Fig. 0.7). In conjunction with this, selection plants delayed flowering by approximately 4 days, had an extended life cycle, and produced 34% more total biomass than control plants (Fig. 0.7). Initially, no differences in physiology were detected between selection and control plants (Ward *et al*., 2000). However, in a more recent study with the same populations (and improved methods), Gonzalez-Meler *et al*. (2009) found that respiration rates were lower in *Arabidopsis* plants selected at 200 ppm CO_2 than in control plants (without reductions in N tissue content), with a large portion of this response occurring from reduced activity of the alternative pathway. Reduced respiration rates improved the carbon budgets of the low [CO_2]-selected plants, representing a potentially important adaptive response to low [CO_2]. Also with respect to the Ward *et al*. (2000) study, Bone & Farres (2001) surveyed the literature and found that the rate of selection at low [CO_2] was among the fastest rates reported for a novel global change factor based on both rates of changes over time (years) and generation number. Taken together, these studies show that low

[CO_2] can act as a strong selective agent on C_3 plants, and suggest that single generation studies may not be fully representative of the responses of glacial plants to low [CO_2].

Using fossil plants from different regions, a variety of studies have shown that ancient plants exhibited higher stomatal density/index during low [CO_2] periods of the past (see Beerling & Chaloner, 1993; Beerling & Woodward, 1993; Wooler & Agnew, 2002; Roth-Nebelsick, 2005). A number of groups have stated that this may be an adaptive response to low [CO_2] that reduced the resistance for entry of CO_2 into the interior of leaves when [CO_2] was most limiting, but potentially at the cost of higher water loss. Other groups, however, have not found a correlation between stomatal density/index and [CO_2], and have argued against this possibility (see Körner, 1988; Bettarini *et al*., 1998; Maherali *et al*. 2002; and mixed responses in Knapp *et al*., 1994). We will briefly describe the data supporting both sides of this issue, and make some suggestions for possibly resolving this debate.

In support of this idea, Beerling *et al*. (1993a) reported higher stomatal density and index of European *Salix herbacea* L. that occurred during low [CO_2] of the LGM relative to modern plants (Fig. 0.8). In addition, using specimens from packrat middens collected from the Great Basin, Van de Water, *et al*. (1994) found that stomatal density was 17% higher in *Pinus flexilis* needles dating to the LGM relative to the Holocene; it is important to note however, that in this study, higher stomatal density did not translate into higher c_i/c_a (lower, in fact), possibly because of reduced g or changes in photosynthetic capacity. In addition to these findings, the relationship between stomatal density/index and [CO_2] has been supported by reductions in stomatal density/index during the contemporary rise in [CO_2], and such responses may serve to conserve water as CO_2 becomes less limiting (Woodward, 1987; Beerling *et al*., 1993a). More specifically, Beerling (2005) found that U.K. populations of *Selaginella selagenoides* and *Selaginella*

kraussiana exhibited an approximate 30% decrease in stomatal index between the period when [CO_2] rose from 280 to 360 ppm. Interestingly, Wagner *et al*. (1996) looked at this issue from an acclimation perspective, and showed that stomatal density decreased within the lifetime of a single birch tree (*Betula pendula*) during the contemporary rise in atmospheric [CO_2] (stomatal density declined -0.6% for every 1 ppm increase in [CO_2]). This group considered this a reflection of evolved plasticity that may have emerged from changing [CO_2] over geologic cycles.

Although many studies find evidence for higher stomatal density (or index) at low [CO_2], approximately 12% of studies find the completely opposite trend (Royer, 2001), and this may be an underestimate since negative results are usually more difficult to publish. For example, in a study of modern C_3 grassland species (*Solanum dimidiatum*, *Bromus japonicas*) grown across a [CO_2] gradient (200-550 ppm), plants exhibited lower stomatal density at low [CO_2] (Maherali *et al*. 2002), and instead had larger stomatal pore size, suggesting an alternative response to limiting [CO_2]. In addition, Körner (1998) found no relationship between stomatal density and [CO_2] for 200 plant species from central Europe that grew during the pre-industrial period (270 ppm [CO_2]) versus modern times (340 ppm [CO_2]), including both alpine and lowland species. Royer (2001) also pointed out that most studies supporting the notion of higher stomatal density or index at low [CO_2] occur with fossil plants (88 and 94% of studies, respectively), with fewer studies showing this response in experimental scenarios with modern plants grown for short periods at low [CO_2] (36 and 40% of studies, respectively). This may be indicative of an evolved response that occurs only after many generations at low [CO_2] as suggested by Royer (2001), and/or may reflect the fact that other growth conditions (water, light, temperature) are less controlled in fossil studies than in modern experimental work. In opposition to Royer's idea,

Bettarini *et al*. (1998) studied 17 plant species growing near high [CO_2] springs in Central Italy where [CO_2] was twice the modern value for at least two centuries, and found that stomatal density was unaffected by elevated [CO_2] in the majority of species.

Despite these inconsistencies, the issue of stomatal index/density response to [CO_2] of the past has increased interest and investment in the understanding plant responses of low [CO_2]. Roth-Nebelsick (2005) laid out the challenges to this field, including high variance in stomatal measurements, especially in fossil samples, large interspecific differences, non-linear response to [CO_2] increase, and differences between long-term and short-term studies. To date, a specific developmental mechanism linking stomatal index/density to [CO_2] has not been determined, although the *Arabidopsis* HIC (high carbon dioxide) gene may play a role in this response (Gray *et al*., 2000). The authors suggest that these challenges may be addressed by striving to identify fossil material that is growing under more tightly controlled conditions for this work (i.e. limiting differences in factors other than [CO_2]), as well as advancing the understanding of molecular mechanisms tied to this response (see Bergmann & Sack, 2007). One approach may be to conduct a thorough QTL (quantitative trait loci) analysis with model plants to determine the chromosomal regions (and eventually genes) that influence stomatal density/index across a range of [CO_2] growth conditions. A more thorough understanding of the primary mechanisms driving stomatal density/index responses to [CO_2] may eventually allow us to resolve the reasons for the inconsistencies reported in this field.

In addition to studying leaf morphology, researchers have also analyzed the carbon isotope ratios of fossil material to determine if there were evolutionary shifts in leaf physiology during low [CO_2] periods (Beerling *et al*., 1993b; Beerling, 1996; Van de Water *et al*., 1994; Pedicino *et al*., 2002; Ward *et al*., 2005). For example, Van de Water *et al*. (1994) calculated

c_i/c_a from carbon discrimination values (that account for changes in carbon isotope ratios of source air through time) of *P. flexilis* leaves that occurred between the LGM and the Holocene. They found lower c_i/c_a during the last glacial period (0.55) relative to the Holocene (0.64), which was the result of either lower g or higher photosynthetic capacity. Most interesting here was the finding that neither c_i/c_a nor c_a-c_i (representing the $[CO_2]$ gradient) were maintained through time, and c_i values dropped as low as 109.7 ppm during the last glacial period. Ward *et al*. (2005) also calculated c_i/c_a from carbon discrimination measurements in *Juniperus* trees from the Rancho La Brea tar pits in southern California. The authors found that c_i/c_a ratios were actively maintained over 60,000 years (as hypothesized by Ehleringer & Cerling, 1993), and as a result, glacial trees experienced c_i values as low as 113 ppm CO_2. If such reductions in c_i were to occur in modern vegetation, plant growth would be reduced by half, according to studies with modern plants (Polley *et al*., 1993a; Van de Water *et al*., 1994; Ward *et al*., 2005). As a whole, these studies support the notion that trees were potentially carbon starved during low $[CO_2]$ periods because of c_i values that are for the most part unprecedented in modern vegetation.

VII. Interactions of low $[CO_2]$ with other factors

A variety of studies have examined the interactive effects of low $[CO_2]$ with other environmental factors such as temperature, water, and nutrients. It is expected that low $[CO_2]$ would accentuate the effects of other stressors, mainly because plants would already be compromised by low carbon availability (Cowling & Sage, 1998; Cowling & Sykes, 1999; Ward, 2005). Certainly the interactions of low $[CO_2]$ with other environmental factors may have had synergistic effects on physiology, plant productivity, and evolution. To address this issue,

recent studies incorporating the interactive effects of low [CO_2] with temperature, water, and nutrients are described below.

1. Temperature

Sage & Cowling (1999) studied the interactive effects of low [CO_2] and high temperature on several C_3 species (*P. vulgaris*, wheat, and tobacco). They found that at high temperature (35 °C day/ 29 night °C), biomass production was reduced by 75-95% at 200 ppm CO_2 relative to 380 ppm CO_2, whereas at low temperature (25 day °C / 20 night °C) biomass production was only reduced by 40-60%. Interestingly, the effects of high temperature on the low-[CO_2] response could not be attributed to lower A (or indirectly, higher photorespiration), but rather to reduced leaf area (relative to total mass) that resulted from lower leaf expansion and less leaf initiation (Sage & Cowling, 1999). From this and other similar studies, altered leaf development has been implicated as the primary response to high temperature stress at low [CO_2]. Moreover, these results suggest that C_3 plants that occurred in warm regions, such as in the tropics, may have been most negatively affected by low [CO_2] during glacial periods.

In a more recent study, Ward *et al*. (2008) grew *A. theophrasti* at low [CO_2] (200 ppm), with both modern (30/24 °C) and glacial temperatures (22/16 °C). The authors discovered that any beneficial effects of low temperature on the C_3 species, such as reduced photorespiration, did not overcome the very negative effects of low [CO_2] on growth. Also, the authors could not detect an effect of temperature on physiology (A, respiration, g), biomass production, or total leaf area at low [CO_2]. This may be explained by earlier empirical work (using *Nerium oleander*) showing tht the sensitivity of C_3 photosynthesis to increasing leaf temperature is diminished at low [CO_2] (Berry & Björkman, 1980; see Fig. 4 therein). In the same study, Ward *et al*. (2008)

found that C_4 plants (*Amaranthus retroflexus*) were greatly inhibited by the lower glacial temperatures, but still maintained a large absolute growth advantage over the C_3 species in response to low [CO_2].

2. Water

It has been predicted that low [CO_2] during glacial periods would have increased the water consumption of C_3 plants, and this has been supported experimentally, mainly through single generation studies (Baker *et al*., 1990; Polley *et al*., 1992; Beerling & Woodward, 1993; Polley *et al*., 1993a,b; Sage, 1995; Ward *et al*., 1999; Tonsor & Scheiner, 2007). Higher water loss is often a result of greater leaf biomass and/or area relative to total plant mass (proposed by Sage & Coleman, 2001; shown in Dipperty *et al.*, 2005; Ward *et al.*, 1999 in well-watered plants; also see Medrano *et al.*, 1995 where this response occurred during selection at low [CO_2]), higher g, and greater stomatal density that enhance CO_2 uptake on a whole-plant basis, but produce greater water loss. It is worth noting that despite evidence indicating greater water demand of plants at low [CO_2], precipitation was much lower during glacial periods on a global basis (Yung *et al.*, 1996; Lambert *et al.*, 2008). Such a combination of low water availability and low [O_2] may have been extremely stressful on plants and may have interacted to greatly reduce productivity during glacial periods.

In one example of work investigating this issue, Ward *et al*. (1999) grew both C_3 (*A. theophrasti*) and C_4 (*Amaranthus retroflexus*) species at 180 (glacial), 270 (pre-industrial), 350 (modern), and 700 (elevated) ppm CO_2 with severe drought treatments. In this case, the authors found that C_3 species responded to drought by dropping a large number of leaves, and retaining high water potential in remaining leaves at all CO_2 treatments. At 180 ppm CO_2, however, C_3

plants retained relatively greater leaf area (by dropping fewer leaves) and delayed the lowering of g following the induction of drought relative to plants grown at 350 ppm CO_2. The combined effects of these responses contributed to the maintenance of a positive carbon budget in C_3 plants grown at 180 ppm CO_2. Surprisingly, the response of C_3 species produced similar degrees of relative recovery from drought at low [CO_2] (assessed as biomass before and after drought) as in the C_4 species. Thus, developmental and growth responses to low [CO_2] and drought superseded the effects of physiological responses in this case, producing similar relative recovery of C_3 and C_4 plants that would not have been predicted from theoretical expectations. Note, however, that in this study (Ward *et al*., 1999) the C_4 species still had much higher biomass on an absolute basis at low [CO_2] compared with the C_3 species.

In the same study (Ward *et al.*, 1999), the C_4 species (*A. retroflexus*) grown under well-watered conditions responded physiologically to changes in [CO_2] with respect to water use. With increasing [CO_2] between 180 and 700 ppm CO_2, the C_4 species showed gradual increases in A and decreases in g and transpiration that somewhat mimicked the responses of the C_3 species. These physiological responses, however, did not result in enhanced leaf area and biomass production, but did serve to conserve water. *Amaranthus* was previously shown to reach CO_2 saturation at a c_i value of 200 ppm CO_2 (Tissue *et al.*, 1995), indicating that this species may not have been CO_2-saturated at the low-CO_2 treatment (180 ppm), explaining why it may have responded physiologically to increasing [CO_2]. Similar to these findings, Polley *et al.* (1994) observed that *Schizachyrium scoparium* (C_4) exhibited decreased g and had higher A between 200 and 340 ppm CO_2, but plant growth remained unaffected. In addition, lower g and higher A have been observed in *A. retroflexus* (C_4) and *Setaria faberii* (C_4) in response to CO_2 enrichment (Garbutt *et al.*, 1990). Also, when grown in competition, elevated [CO_2] stimulated

the biomass production of *A. retroflexus* (C_4) to a greater extent than *A. theophrasti* (C_3) (Bazzaz *et al.*, 1989). These results, as well as others (Cunniff *et al.*, 2008), demonstrate that some C_4 species can be quite physiologically responsive to changes in [CO_2], suggesting that, like C_3 plants, C_4 species may have also had higher water demands during the LGM than in modern times.

3. Nutrients

Very little work has focused on the interactive effects of low [CO_2] with nutrients. As mentioned above (section V.1.), Polley *et al*. (1995) found that across a [CO_2] gradient (200-360 ppm; without specific N treatments), WUE was decreased much more consistently at the lowest [CO_2] (200 ppm) than was NUE among C_3 annual grasses and a woody perennial. This indicated that the requirements for water and nitrogen may have shifted between the LGM and modern times, with higher water demand appearing to be the more dominant and consistent factor. In a newer study with *Populus deltoids* (cottonwood), Lewis *et al*. (2010) showed that low [CO_2] (200 ppm) inhibited the responses of A and biomass production to increases in phosphorus (P), whereas positive responses to P additions were more prominent at modern (350 ppm) and elevated [CO_2] (700 ppm). Even more interesting was the finding that at high P concentrations (0.5mM), biomass was less enhanced with increases in [CO_2] below the modern value relative to above, reversing the trend for previous studies that did not include P treatments (see section V.2.). In limiting P scenarios, Campbell & Sage (2006) found that white lupin (*Lupinus albus* L.) was restricted in its ability to respond to increasing [CO_2] above 200 ppm with respect to A. As a result of these findings, the authors emphasized that nutrient availability must be factored into

models predicting responses of ancient plants to low [CO_2], as well as plant responses to increases in [CO_2] between pre-industrial and modern times.

Sage (1995) predicted that symbiotic relationships (mychorrizal fungi, N fixing bacteria) may have been less prevalent during glacial periods due to low [CO_2]. The high investment of photosynthate required to support symbionts may not have been as beneficial during a period when carbon was potentially more limiting than nutrients. In support of this idea, Polley *et al*. (1994) found that N fixation was reduced for plants (*Prosopis glandulosa*) grown at low [CO_2] compared with modern [CO_2] with the same availability of soil N. Again, this speaks to the potential for major changes in ecosystem functioning as a result of shifts in limiting resources during low [CO_2] periods.

VIII. Low [CO_2] effects on community composition

Past work has incorporated low [CO_2] effects on community composition, with a focus on shifts in plants possessing the C_3 versus C_4 photosynthetic pathways. From stable carbon isotope ratios, it is clear that low-[CO_2] periods were characterized by major shifts from C_3 to C_4 dominance in some regions (Ehleringer *et al.,* 1991; Cerling *et al.,* 1997; Street-Perrott *et al*. 1997; Cerling *et al.,* 1998; Flores *et al*., 2009). Other studies, however, have concluded that climate (temperature, precipitation) may be a stronger force behind C_3-C_4 shifts relative to low [CO_2] (Morgan *et al.,* 1994; Latorre *et al.,* 1997; Pagani *et al.,* 1999; Huang *et al.,* 2001; Keeley & Rundel, 2005; Huang *et al.,* 2006; Flores *et al*., 2009; Edwards *et al.*, 2010). Many of these discrepancies may be explained by the regional conditions of each study, and whether or not water availability and temperature further enhanced or negated the transition to C_4 dominance.

Past studies have also investigated ancient shifts in plant communities in response to low [CO_2] via empirical studies with pollen records, as well as modeling efforts. Using a process-based vegetation model, Jolly & Haxeltine (1997) predicted a dominance of xerophytic scrub in East Africa during the LGM compared with the current montane forest. In this case, modeled results showed that lowering [CO_2] to the LGM value of 190 ppm produced a shift to the LGM scrub community, even under modern temperature and precipitation values (and may explain the enhanced African scrub belt from LGM pollen records). For this reason, the authors concluded that paleoclimate reconstructions based on present climate analogs may be unreliable if they do not incorporate the direct effects of low [CO_2] on vegetation.

In a different study, Levis *et al*. (1999) predicted that low [CO_2] of the past 21,000 years favored grasslands and tundra over the forests currently located at temperate and boreal latitudes. These changes would have greatly affected evapotranspiration and possibly the entire water cycle of the region. This result suggested that large-scale changes in the characteristics of canopy cover (e.g. leaf area index, LAI) in response to low [CO_2] may have had a more overwhelming effect on ecosystem functioning than smaller-scale physiological changes within individual plants (Levis *et al*., 1999). Furthermore, Cowling *et al*. (2008) modeled vegetation distributions in central Africa during the LGM and found that even though tropical broadleaf forests were not displaced during the LGM (with the exception of grassland encroachment on the edges), structural changes in forest canopy characteristics (e.g. lower LAI, tree height, and density) may have greatly altered ecosystem functioning. This change may have further impacted the migration patterns of numerous plant and animal species, including *Homo sapiens*. Taken together, these studies show that low [CO_2] can influence the characteristics of community

structure, ranging from alterations in canopy characteristics, shifts in C_3 versus C_4 dominance, to changes in biome structure.

IX. Low [CO_2] effects on the ecosystem

In order to better understand the effects of low [CO_2] on whole ecosystems, global climate and biosphere models have been used to predict glacial values of NPP, terrestrial carbon storage, and isoprene emissions. The following section summarizes the results of these modeling efforts and discusses the benefits and limitations of scaling low-[CO_2] effects on individual plants to the ecosystem level.

Using a mass-balance carbon isotope approach, Bird *et al*. (1994) constrained the increase in terrestrial carbon storage between the LGM and pre-industrial period to 310-355 Gt (or 270-720 Gt when oceanic $\delta^{13}C$ uncertainties were incorporated), which supported previous estimates made by others using different approaches (e.g. Friedlingstein *et al*., 1992; Prentice *et al*., 1993; Van Campo *et al*., 1993). Later, Francois *et al*. (1998) coupled the CARAIB vegetation model to a general circulation model (ECHAM) in order to understand changes in terrestrial NPP and carbon storage across glacial-interglacial boundaries. Their modeling efforts predicted that NPP values increased from 38 Gt C per year during the LGM to 53 Gt C per year during the pre-industrial period; in addition, their estimates of changes in carbon storage overlapped that of Bird *et al*. (1994). Francois *et al*. (1998) attributed low NPP during the LGM to changes in land area, cooler temperatures, and effects of low [CO_2] on vegetation. In a subsequent analysis, they predicted that low-[CO_2] effects on vegetation were the dominant factor in reducing NPP during the LGM, and a later sensitivity analysis confirmed this finding (Otto *et al*., 2002; Francois *et al*. 2006). In addition, Turcq *et al*. (2002) found that carbon storage in Amazonia ranged between 44

and 94% of modern amounts, and this reduction was attributed to the effects of low [CO_2] on vegetation, as well as cooler and drier conditions.

Harrison and Prentice (2003; using BIOME4) predicted that the effects of low [CO_2] on plants reduced tropical forest cover by 44-69% during the LGM. Before this, Levis *et al*. (1999) also predicted that increases in C_4 grasslands during the LGM, which occurred at the expense of forests, produced positive feedbacks associated with albedo that resulted in warmer and drier ecosystems, particularly in the tropics. Using a global-vegetation model, Crucifix *et al*. (2004) also found indications of major changes in vegetation distribution between the LGM and the pre-industrial period, with specific disappearance of the Siberian boreal forest, greater shrub cover in Europe, and an expansion of subtropical desert land area. Much of this change was attributed to differential effects of low [CO_2] on plant physiology and water use, where grasses and shrubs had lower water requirements during glacial conditions than trees. By comparing modeled estimates to empirical continent-scale palaeoclimate data, Jolly & Haxeltine (1997) stated that efforts to determine paleo-temperatures based on tree-line depression may be faulty, and may over-estimate reductions in temperature if low-[CO_2] effects on vegetation are ignored. Taken together, these studies support the recent plea by Prentice & Harrison (2009) to more widely incorporate direct physiological effects of low [CO_2] on plants when predicting past climates and ecosystem functioning, because failure to do so may result in erroneous conclusions.

Global vegetation models have also been used to better understand how changes in [CO_2] may have affect post-fire recovery rates of trees and grasses from the LGM through modern times. In South African grasslands, Bond *et al*. (2003) predicted from modeling efforts that low [CO_2] of the LGM reduced sapling growth to the point where young trees could not reach a fire-proof size between fire events, resulting in the elimination of trees in favor of C_4 grasses.

Furthermore, increased [CO_2] during the Holocene may have allowed for the reappearance of savanna trees (Bond *et al*., 2003). Moreover, this study predicts that the interactive effects of low [CO_2] and disturbance factors may have altered plant establishment patterns, which is an issue that had not been previously appreciated.

Plant emissions of trace gases can be influenced by changes in [CO_2], climate, and other environmental factors, and can also feed back to influence climate change through the production of organic aerosols (Possell *et al*., 2005). In particular, isoprene emissions constitute approximately half of the biogenic volatile organic compounds released by the terrestrial biosphere, and therefore should be incorporated into the glacial-interglacial transition. To address this issue, Possell *et al*. (2005) grew the known isoprene-emitters *Mucuna pruriens* and *Arundo donax* in growth chambers at glacial (180 ppm), pre-industrial (280 ppm), and modern (366 ppm) [CO_2]. On a leaf area basis, the lowest CO_2 treatment produced a three-fold increase in isoprene production relative to modern [CO_2]. However, low [CO_2]-induced reductions in above ground biomass and LAI offset this increase, such that total canopy isoprene emissions showed no significant change between low and modern [CO_2]. When cooler conditions of the LGM were incorporated, isoprene emissions were significantly reduced at the canopy level and on a leaf mass basis, and remained similar across all CO_2 treatments. In a more recent study, Wilkinson *et al.* (2009) showed the Eucalyptus trees had significantly higher isoprene emissions at 240 ppm than at 380 pm, while sweetgum showed no significant change across this gradient It is still not clear why plants exhibit differential isoprene emissions in response to low [CO_2] or even how best to represent these changes in modeling efforts. This emphasizes the need to better understand the interactive effects of climate and [CO_2] on trace gas emissions of the past.

On a different note, little is known about how reduced [CO_2] may have influenced the availability and quality (e.g. C/N) of food sources to herbivores and how shifts in vegetation may have affected animal diets during glacial periods (Ehleringer *et al*., 2002). The majority of studies dealing with these issues are mainly focused on other time periods such as the Cenozoic (MacFadden, 2005) and late Miocene-Pliocene boundary (Cerling *et al*., 1997). Hopefully in the future, those with animal expertise will work to better understand the potential consequences of low [CO_2] effects on glacial animals.

As outlined above, a variety of modeling studies show strong evidence for reductions in NPP and carbon storage during glacial periods, with low [CO_2] playing a major role in these changes. Much of this modeling work relies on empirical and theoretical changes in A and g with [CO_2], mainly from Farquhar *et al*., (1980). As mentioned above, there tends to be a close correlation between A and growth (and possibly reproductive output) at low [CO_2]. Therefore, the sole use of physiological data for estimating glacial NPP and carbon storage has validity, and is probably accurately representing our understanding of single generation responses of modern plants to [CO_2] of the past. It is quite possible, however, that as we gain further insight into our understanding of plant evolutionary responses to low [CO_2], that these large-scale estimates may need to be revised. As illustrated earlier (section VI.), Ward *et al*. (2000) found a 34% increase in plant biomass at 200 ppm CO_2 following only five generations of selection for high fitness. Unfortunately, very few estimates of this kind exist. Thus, as we learn more about potential adaptive responses to low [CO_2], these should be incorporated into future modeling efforts.

X. Low [CO_2] effects on early human societies

The emergence of agriculture among early human societies appeared throughout the world, between 5,000 and 10,000 years ago, and this represents a rather short time span with respect to evolutionary change, particularly for crop plants. Recently, Sage (1995) proposed that such synchronous origins may have been the result of a common global factor, more specifically, the rise in [CO_2] from 200 to 270 ppm that occurred between 15,000 and 10,000 years ago (Fig. 0.9). In this section, we discuss the debate over the possible drivers for the origin of agriculture, with a specific emphasis on the role that low [CO_2] may have played in the emergence of agriculture.

In the past, anthropological literature had mostly attributed the onset of agriculture to changes in human social and cultural structure and complexity (Bar-Yosef, 1998; Mannion, 1999). Although this has been a long-standing idea, cultural mechanisms were unlikely to have occurred simultaneously throughout all societies, and therefore this idea does not fully explain the synchronous emergence of agriculture (Cunniff *et al*., 2008). Other ideas have incorporated the effects of changing palaeoclimates, such as increasing precipitation and temperature (Bar-Yosef, 1998), although often, these are in the context of how climate impacted human society and not crops themselves (Bar-Yosef, 1998). Alhough changes in climate are influential in their impact on human culture, a sole focus on this aspect of environmental change ignores the potentially large direct effects of [CO_2] on crop plants.

A plethora of past work has shown that the increase in [CO_2] that occurred between 15,000 and 10,000 years ago may have been large enough to have had a profound impact on crop productivity, and hence on human subsistence patterns (Sage, 1995; Fig. 0.9). In general, glacial conditions would have been a hostile environment for C_3 crops because of low [CO_2], as well as

drier soils and higher seasonal variation (Richerson *et al*, 2001). As the interglacial period commenced, the onset of rising [CO_2] and other climatic changes would have removed an environmental limitation to the development of agriculture (Sage, 1995). Increasing [CO_2] during the interglacial period may have directly enhanced plant productivity and may have reduced the effects of interactive stressors, such that crop production could be sustained year after year within human societies (Sage, 1995).

Anthropologists are beginning to incorporate the importance of CO_2 into their ideas on agricultural development. For example, Bettinger *et al*. (2009) attributed the development of agriculture to the combined effects of climatic and cultural changes, including increasing [CO_2]. He commented that once the external environmental constraints were removed, social innovation and more organized human communities became the predominant driver for the development of agriculture (Bettinger *et al.*, 2009).

The importance of [CO_2] in agricultural origins has met with some debate mainly since some early crops were C_4 species (although these were in the minority), and it was initially thought that they would not have responded to increasing [CO_2] (Cunniff *et al*., 2008). By contrast, however, a number of studies have shown that C_4 species experience up to a 40% increase in biomass production between glacial and interglacial [CO_2], as well as a 35% reduction in g, indicating that C_4 species may have been strong responders to this rise in [CO_2] (Cunniff *et al*., 2008). In addition, increasing WUE and A with rising [CO_2] would have been most pronounced in the hot climates where development of C_4 crops first occurred (e.g millets in north China, sugarcane in New Guinea). Future research may be able to tease apart the impacts of [CO_2], climate change, and cultural development on the emergence of agriculture among

different societies. Such work will be enhanced by stronger collaborations among anthropologists, plant physiologists, and ecologists.

XI. Conclusions

From the studies highlighted in this review, as well as others, we now know that the influence of low [CO_2] during glacial periods transcends many levels ranging from physiological effects on individual plants to changes in ecosystem functioning, and may have even influenced early human agricultural development. Results of these studies provide a baseline for plant response to minimal [CO_2] that occurred during the evolution of land plants. Within a single generation of exposure to low [CO_2], modern C_3 plants show an average reduction in photosynthesis and biomass production of 50% (although this can range from 40-70% among species/genotypes). When scaled up in space and time, these physiological responses imply large reductions in NPP and carbon storage during glacial periods relative to the present, as well as influences on vegetation distributions, post-fire recovery, and trace gas emissions.

Despite these advances, we still have much to learn with respect to plant evolution at low [CO_2]. Although numerous environmental factors were changing between glacial and interglacial periods, it is clear that CO_2 alone can act as a strong selective agent on plants. For example, productivity increased by 34% within a C_3 model system (*Arabidopsis)* after only five generations of selection for high fitness at low [CO_2] (Ward *et al*., 2000). The potential for rapid genetic change in response to low [CO_2] is great and carries implications for our understanding of ancient plant productivity and ecosystem functioning. For these reasons, an improved understanding of plant adaptation to changes in [CO_2] is necessary to accurately predict plant

function under both past and future conditions, and such information must eventually be incorporated into modeling efforts.

Overall, a better understanding of plant responses to low [CO_2] will help us to understand how plants acclimated and adapted to changing carbon resources over geologic time scales. In order to advance our understanding in this area, it will be necessary to develop larger and more realistic growth environments, include more disciplines in order to move this field forward, extend experimental durations to allow for full acclimation and adaptation of plants, and continue studying the interactions between low [CO_2] and other environmental factors. Moreover, this will allow us to better predict future plant responses to anthropogenic increases in [CO_2], since changes in plants that were a result of low-[CO_2] periods of the past may still influence the potential for plants to fully utilize increasing carbon resources in the future.

Introduction Figures

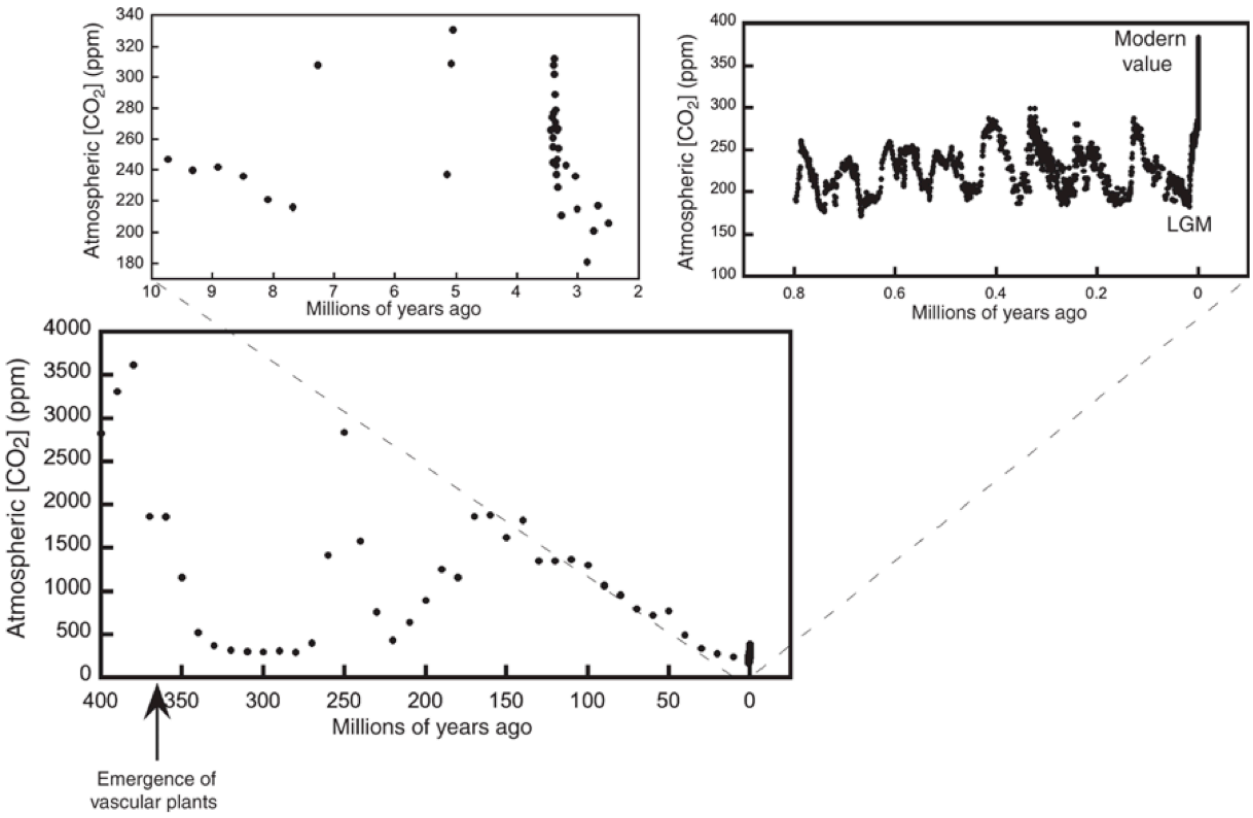

Figure 0.1. Changes in atmospheric [CO_2] throughout the evolution of vascular land plants. The upper right insert shows the past million yr expanded in order to show low [CO_2] during glacial periods. The upper left insert is expanded to show low [CO_2] periods over the last 10 million yr (data are from Petit *et al.,* 1999; Monnin *et al*., 2001; Siegenthaler *et al*., 2005; Berner, 2006; Lüthi *et al*., 2008; Keeling *et al.*, 2005; Tripati *et al*., 2009).

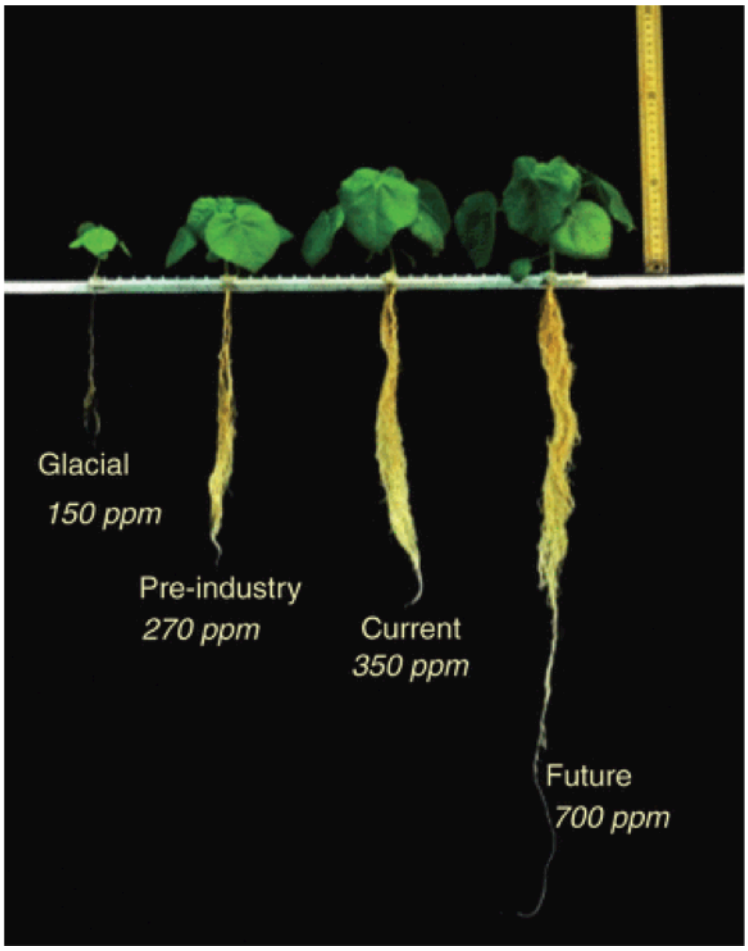

Figure 0.2. Representative plants of *Abutilon theophrasti* (C_3) grown at glacial through future [CO_2]. All plants were 14 d of age and were grown under similar water, light, and nutrient conditions. These plants were photographed during a study by Dippery *et al*. (1995). (Photograph is courtesy of Anne Hartley, Florida Gulf Coast University.)

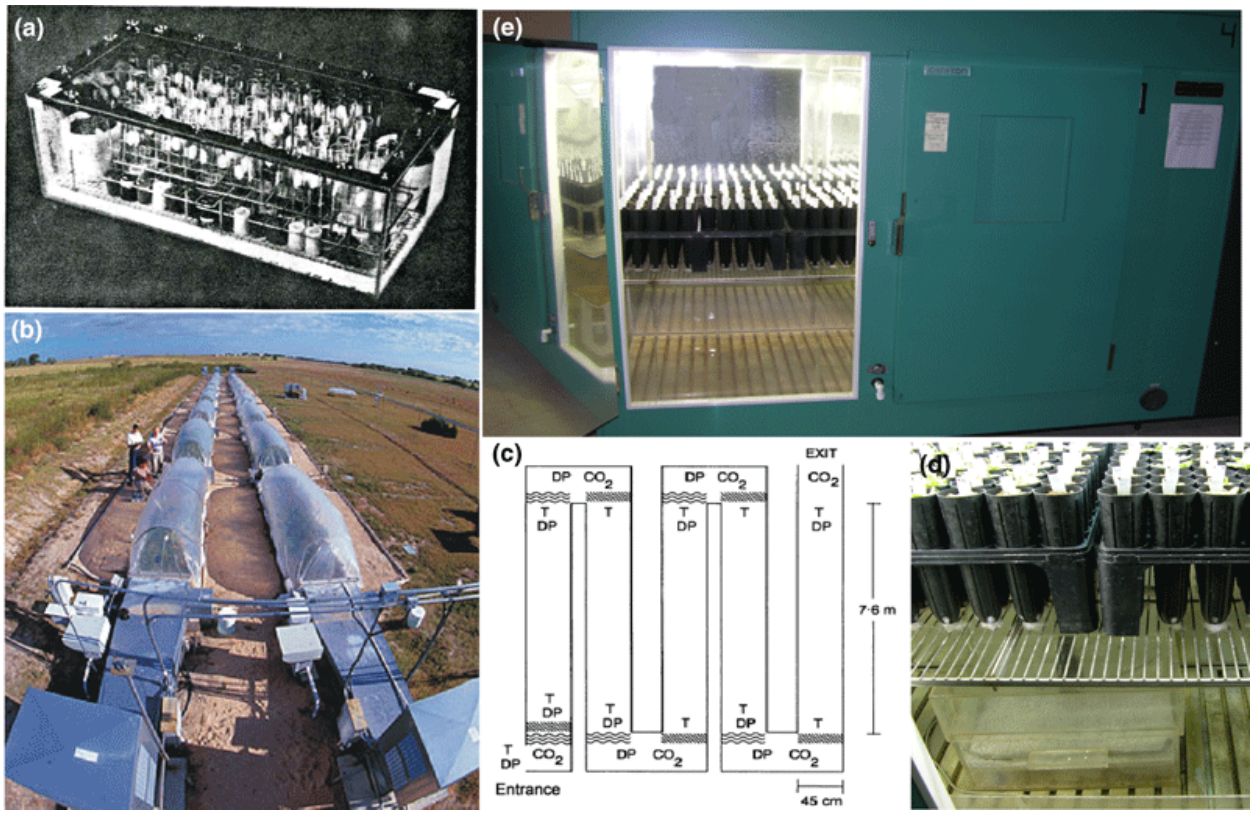

Figure 0.3. Experimental approaches for achieving low-[CO_2] treatments. (a) Small growth chamber with C_4 plants grown side by side with experimental C_3 plants, used by Sharma *et al*. (1979, Fig. 1); (b) tunnel chambers for reducing [CO_2] along a continuum, described by Mayeux *et al.* (1993) (photograph courtesy of Wayne Polley, Grassland, Soil, and Water Research Laboratory); (c) diagram of the tunnel chambers indicating where [CO_2] (CO_2), dew point (DP), and air temperature (*T*) are sampled. Wavy lines represent chilled water cooling coils and cross-hatched areas represent electrical resistance heaters (from Mayeux *et al.*, 1993, Fig. 2); (d) experimental growth chamber with soda lime box underneath plants to reduce [CO_2] within the chamber (photograph courtesy of Joy K. Ward, University of Kansas); (e) reach-in experimental growth chamber with low-[CO_2] control (Conviron BDR-16, photograph courtesy of Joy K. Ward, University of Kansas).

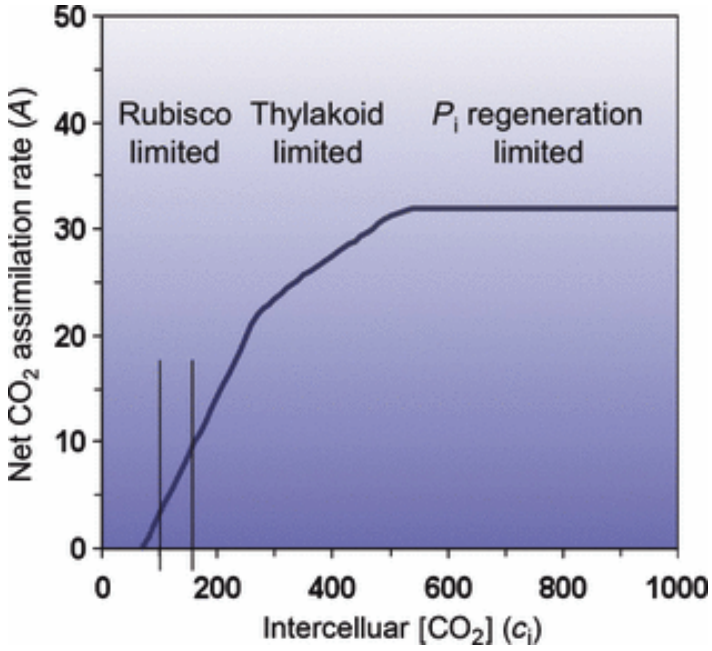

Figure 0.4. Theoretical A-c_i curve showing the relationship between photosynthetic assimilation of CO_2 (A) and leaf intercellular [CO_2] (c_i). The region within the vertical lines represents approximate c_i values experienced by glacial plants (at corresponding atmospheric [CO_2] of 170-200 ppm and based on c_i values from glacial plants; Van de Water *et al.*, 1994; Ward *et al.*, 2005).

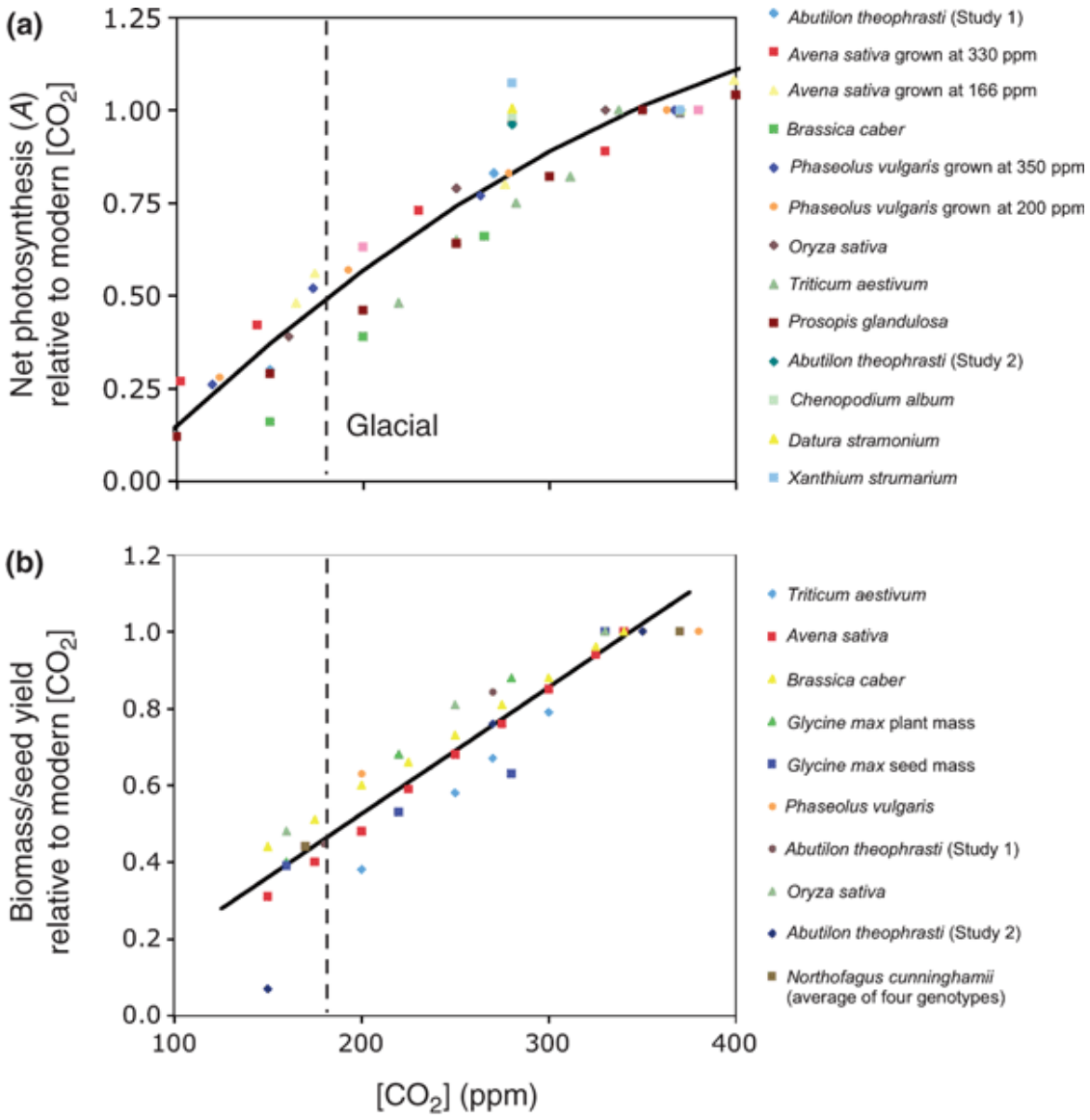

Figure 0.5. Relative net photosynthesis (a) and biomass production (or seed yield) (b) for a variety of modern plants grown at [CO_2] spanning from glacial to modern times. Data in the y-axis are responses at a given [CO_2] relative to modern [CO_2] (350-380 ppm), with the line representing responses for plants grown at the glacial [CO_2] minimum (adapted from Sage & Coleman, 2001, Fig. 3, with updated data from Cowling & Sage, 2998; Ward *et al.*, 1999; Hovenden & Schimanski, 2000; Bunce, 2001).

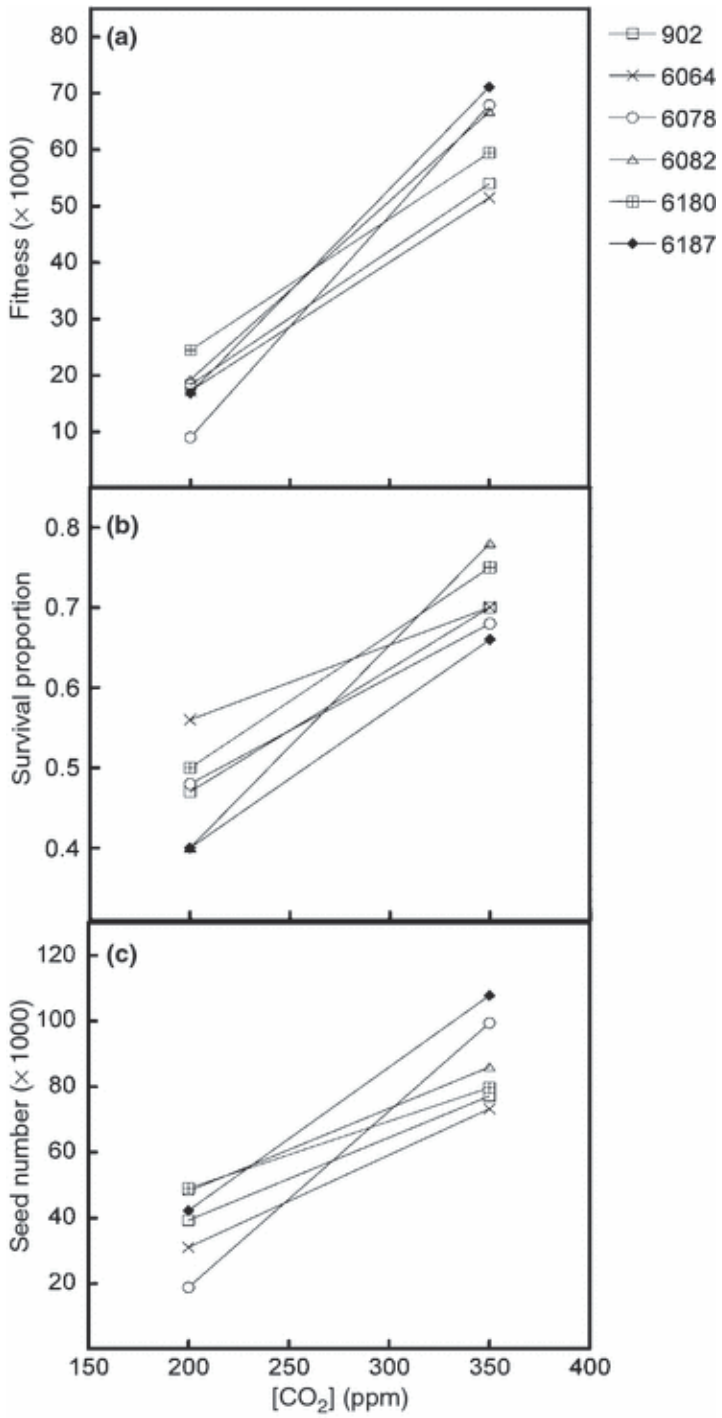

Figure 0.6. Responses of six field-collected genotypes of *Arabidopsis thaliana* grown at 200 and 350 ppm CO_2 and measured for estimated total fitness (a), proportion of survival (b), and total seed number (c). Genotype numbers are from The Arabidopsis Biological Resource Center, Ohio State University (adapted from Ward & Kelly, 2004, Fig. 6).

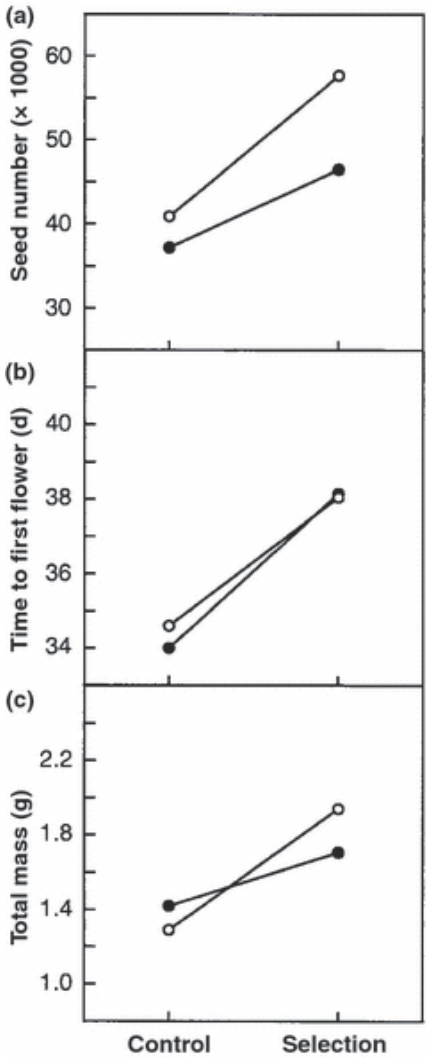

Figure 0.7. Mean values for seed number (a), time (d) to first flower (b), and total biomass (c) for selected and control (nonselected) plants of *Arabidopsis thaliana* at the fifth and final generation of selection for high seed number at 200 ppm. All plants were grown under the same conditions at 200 ppm CO_2. Lines connect mean values for replicate control and selection populations, and open or closed circles designate different growth chambers. Note that low [CO_2] was found to be the selective agent acting on these plants via subsequent reciprocal transplant experiments (see Ward *et al.*, 2000) (adapted from Ward & Kelly, 2004, Fig. 3(a); Ward *et al.*, 2000).

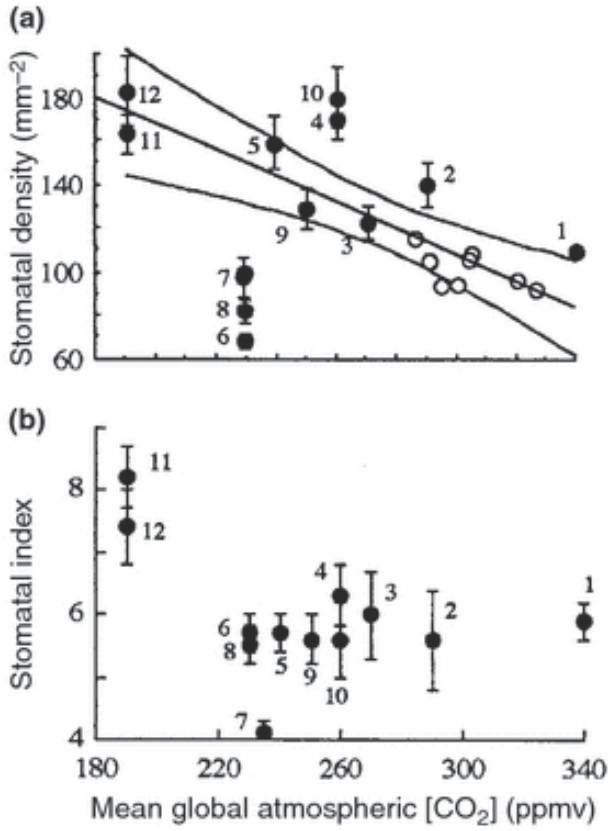

Figure 0.8. Stomatal density (a) and stomatal index (b) of fossil *Salix herbacea* leaves in response to changes in atmospheric [CO_2] from the Last Glacial Maximum to the present (taken from Beerling *et al.*, 1993a, Fig. 2).

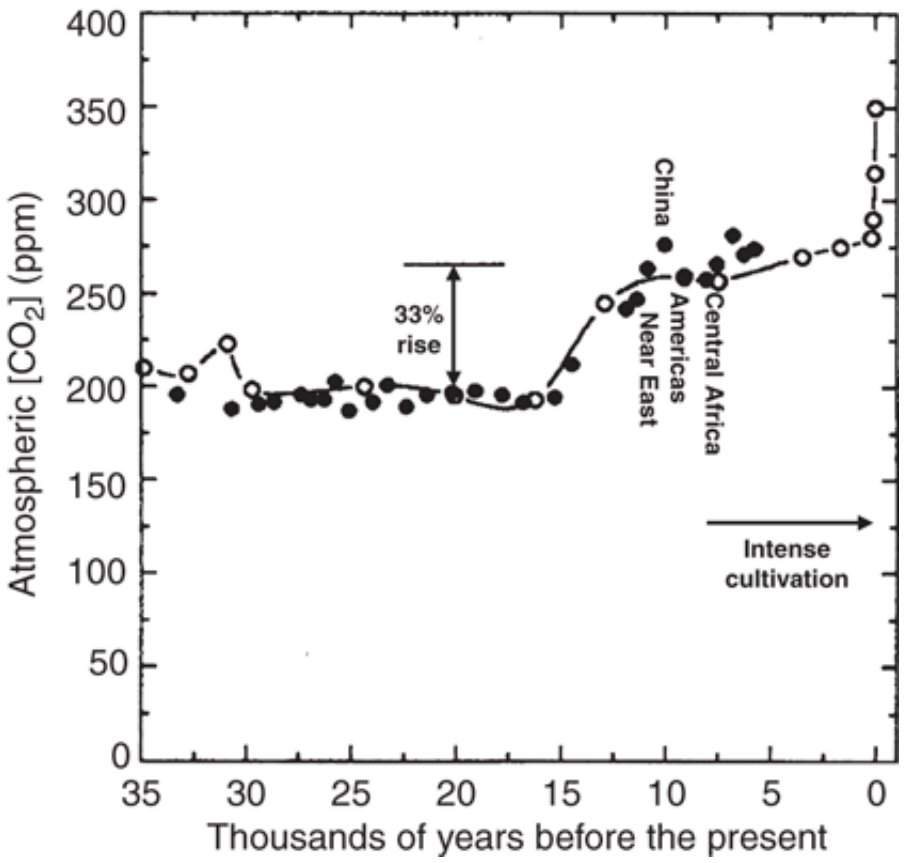

Figure 0.9. The timing for the emergence of agriculture among different human societies and changes in atmospheric [CO_2] through time. Geographic names indicate the period when domesticated plants first appear in the archeological record for that region (from Sage, 1995, Fig. 1).

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Chapter 1: Glacial Trees from the La Brea Tar Pits Show Physiological Constraints of Low CO_2

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Summary

- While studies of modern plants indicate negative responses to low [CO_2] that occurred during the last glacial period, studies with glacial plant material that incorporate evolutionary responses are rare. In this study, physiological responses to changing [CO_2] were compared between glacial (La Brea tar pits) and modern *Juniperus* trees from southern California.
- Carbon isotopes were measured on annual rings of glacial and modern *Juniperus*. The intercellular [CO_2]/atmospheric [CO_2] ratio (c_i/c_a) and intercellular [CO_2] (c_i) were then calculated on an annual basis and compared through geologic time.
- *Juniperus* showed constant mean c_i/c_a between the last glacial period and modern times, spanning 50,000 years. Inter-annual variation in physiology was greatly dampened during the last glacial period relative to the present, indicating constraints of low [CO_2] that reduced responses to other climatic factors. Furthermore, glacial *Juniperus* exhibited low c_i that rarely occurs in modern trees, further suggesting limiting [CO_2] in glacial plants.
- This study provides some of the first direct evidence that glacial plants remained near their lower carbon limit until the beginning of the glacial-interglacial transition. Our results also suggest that environmental factors that dominate carbon-uptake physiology vary across geologic time, resulting in major alterations in physiological response patterns through time.

Introduction

The last glacial period began approximately 110,000 years ago and reached a maximum for global ice volume at 18,000-20,000 years ago. Glacial conditions persisted (except for brief interstadials) until the abrupt transition to the current interglacial period, beginning approximately 14,000 years ago. At the peak of the last glacial period, atmospheric CO_2 concentrations ($[CO_2]$) ranged between 180-200 ppm, which are among the lowest levels that occurred during the evolution of land plants (Berner, 2006; also see Pagani *et al.*, 2009 for an account of similarly low levels ~15 mya). When grown at glacial versus modern $[CO_2]$, modern C_3 plants show 40-70% reductions in photosynthesis and biomass production (Polley *et al.*, 1993; Sage & Coleman, 2001), 20-30% lower survival (Ward & Kelly, 2004), and may even fail to reproduce (Dippery *et al.*, 1995). This is a result of reduced CO_2 substrate concentrations at carboxylation sites, as well as higher photorespiration rates. However, even at reduced paleo-temperatures where photorespiration is decreased, plants are still unable to overcome the severe, negative effects of low $[CO_2]$ (Ward *et al.*, 2008). Such pronounced effects originating at the level of autotrophic physiology have been modeled at the ecosystem scale, and have been predicted to greatly reduce NPP and carbon storage during glacial periods (Turcq *et al.*, 2002; François *et al.*, 2006;). Admittedly, however, modern plants are often grown in glacial conditions for only a single generation, and therefore do not reflect evolutionary responses to low $[CO_2]$. This realization prompted our recent studies of glacial *Juniperus* (juniper) trees that were fully preserved within the La Brea tar pits in southern California (Los Angeles), and that had tens of thousands of years to adapt to low $[CO_2]$. Analysis of stable carbon isotope ratios of complete wood samples (that integrate all tree rings from a given individual) show severely reduced internal $[CO_2]$ (c_i) during the last glacial period that are unprecedented in modern equivalents,

strongly suggesting the existence of major carbon limitations on tree physiology (Ward *et al*., 2005). Thus, the low [CO_2] of glacial periods likely produced a bottleneck on carbon exchange through reduced transfer of CO_2 from the atmosphere to the biosphere. However, it is still unknown if low [CO_2] presented an over-riding limitation on plant physiology relative to other climatic factors (e.g., water, temperature).

The last glacial period represents an ideal time period for addressing this issue, since climate was more variable on an inter-annual level and [CO_2] was exceptionally low relative to modern times (Mayewski *et al.*, 2004). More specifically, ice cores from Greenland indicate extreme stability of Holocene climate compared to that of the last glacial period (Dansgaard *et al.*, 1993). In addition, Dansgaard-Oeschger cycles, which are periods of rapid and abrupt changes in temperature, dust content, ice accumulation and greenhouse gas concentrations, were more prominent during the last glacial period relative to the Holocene (Broecker, 1994; Roy *et al.*, 1996). These patterns recorded in Greenland ice are also documented in ocean sediment cores from the Santa Barbara Basin (Behl & Kennett, 1996; Heusser, 1998; Hendy & Kennett, 1999; Hendy *et al.*, 2002), approximately 100 km northwest of La Brea, our primary research site. Analyses of these cores show a strong teleconnection between atmospheric trends over Greenland and ocean dynamics off the California coast, identifying synchronous climatic events between the two records over the last 60,000 years (Behl & Kennett, 1996; Heusser, 1998; Hendy & Kennett, 1999; Hendy *et al.*, 2002).

In previous work, we did not have wood specimens that allowed for carbon isotope analysis of individual tree rings. Recent excavations at the La Brea tar pits have now yielded higher quality *Juniperus* specimens, allowing for discernment of individual tree rings. Thus, these wood specimens make an excellent model system for testing the constraints of low [CO_2]

on tree physiology relative to the effects of other climatic factors during the last glacial period. Here we compare long-term responses of tree physiology, as well as inter-annual variation within individuals, between the last glacial period and modern times. In doing so, we find the first evidence that low [CO_2] constrained the physiology of glacial trees, as evidenced by a dampened response to inter-annual climate variability.

Materials/Methods

Site Selection

For this study, glacial trees from the Rancho La Brea tar pits (Los Angeles) were sampled and ^{14}C dated to 14.5 to 47.6 kyr BP, with the majority of specimens dating to the last glacial period. *Juniperus* samples from Rancho La Brea cannot be identified to the species level, though analysis by a wood anatomy expert (Ward *et al.*, 2005) and species distributions indicate these samples are either *J. californica* or *J. occidentalis*. Cores of modern trees were collected from three low elevation sites in the Angeles National Forest (*J. californica*, two trees per site, one core per tree) and three high elevation sites in the San Bernardino National Forest (*J. occidentalis*, three trees per site, one core per tree), which are close in proximity to La Brea. Only modern trees from natural areas with well-drained, non-irrigated soils were sampled. Low elevation sites provided a same-site control for glacial La Brea (with the full suite of environmental changes through time), whereas high elevation sites controlled for lower temperatures and higher precipitation of the last glacial period (see Table 1.1, Heusser, 1998; Daly *et al.*, 2008), allowing for isolation of CO_2 effects. Note that conditions at glacial La Brea were wetter than modern times, which differs from most regions that were drier during the last glacial period. While [CO_2] does not vary with elevation, CO_2 partial pressure decreases in

proportion to total atmospheric pressure. Under modern conditions, partial pressures of CO_2 at high elevation sites are 10-30% lower than at low elevation sites, producing an even more conservative comparison between glacial and modern conditions.

Stable Isotope Measurement

We measured stable carbon isotope ratios on alpha-cellulose from individual tree rings of glacial and modern *Juniperus*. Whole tree rings were analyzed in order to provide an integrated measure of the full annual response. Ring wood was separated under a dissecting microscope and alpha-cellulose was extracted from each ring using the method described by Ward *et al.* (2005). Previous work using this method has documented high purity levels of alpha-cellulose with no indication of asphalt contamination from the tar pits. Purity was based on theoretical O/H ratios (weight percent oxygen/weight percent hydrogen) of 7.79-8.08 for alpha-cellulose, with actual values falling well within this range (8.01±0.02 and 7.97±0.04 for modern and glacial samples respectively, Ward *et al.*, 2005). Because our specific compound reflected high purity levels, we do not believe that diagenetic processes would have influenced our results.

Of the five glacial wood specimens that were available with an adequate number of tree rings, three are trunk specimens while two may either be portions of the trunk, or large branch sections. For modern trees, the 10 rings nearest the center were excluded, as is common on dendrochronological work as the juvenile stage often exhibits altered physiological patterns. Apart from this exception, all available rings in all samples were analyzed. Isotope measurements were performed at the Keck Paleoenvironmental and Environmental Stable Isotope Laboratory (KPESIL) at the University of Kansas. $\delta^{13}C$ values were calculated using the following formula:

$$\delta = R_{sample} / R_{standard} - 1$$

where R is the ratio of $^{13}C/^{12}C$, using belemnite carbonate from the Pee Dee Formation, Hemingway, SC (PDB) as the standard. Data were converted to "per mil" (‰) notation by multiplying δ values by 1000. $\delta^{13}C_{cell}$ was converted to $\delta^{13}C_{leaf}$ using a constant offset of –3.2‰ (Leavitt & Long, 1982; Ward *et al.*, 2005). Carbon isotope discrimination was calculated as:

$$\Delta = \frac{\delta^{13}C_{air} - \delta^{13}C_{leaf}}{1 + \delta^{13}C_{leaf}}$$

Conversion to carbon discrimination is necessary as it incorporates changes in $\delta^{13}C_{air}$ through time. $\delta^{13}C_{air}$ was -0.0066 (-6.6‰) during glacial times, but has decreased in modern times to -0.008 (-8.0‰; Leuenberger *et al.*, 1992). From Δ, c_i/c_a was calculated as

$$\frac{c_i}{c_a} = \frac{\Delta - a}{b - a}$$

where a is the fractionation against ^{13}C due to slower diffusion across the stomata (4.4‰) and b is the fractionation against ^{13}C due to Rubisco (27‰).

For each ring, c_i was also calculated from the c_i/c_a ratio using c_a values. For modern samples, c_a values were obtained from direct atmospheric measurements (Keeling *et al.*, 2009) and the Taylor Law Dome ice core (Etheridge *et al.*, 1996). For glacial trees, c_a values were obtained from the Vostok and EPICA Dome C ice cores (Lüthi *et al.*, 2008). To obtain the appropriate c_a values, ^{14}C ages of glacial trees were first converted to calendar ages in order to coincide with ice core data (Beck *et al.*, 2001). Since atmospheric $[CO_2]$ showed only minimal changes throughout the latter portion of the last glacial period that is encompassed in our study, we are confident that $[CO_2]$ levels corresponding to converted ages are accurate to the actual conditions experienced by glacial trees.

Statistical Analyses

Mean c_i/c_a values for high- and low-elevation modern *Juniperus* were not significantly different despite environmental differences between these locations (0.53 ± 0.05 and 0.53 ± 0.06, p = 0.1, ANOVA), and therefore, the two modern sets were grouped together for comparison to glacial values. Since the variance in c_i/c_a was significantly different between modern and glacial *Juniperus* ($p < 0.0001$), a Welch's ANOVA was used to compare modern and glacial c_i/c_a values that accounts for lack of equivalence of variance.

The coefficient of variation (CV) was calculated for c_i/c_a in both modern and glacial samples. CV provides a measure of dispersion of data around the mean, allowing us to compare variation between groups. CV was calculated as:

$$CV = \frac{s}{\bar{x}}$$

where s is the standard deviation, and $\bar{x}$ is the mean. Data are shown in percentage notation by multiplying CV by 100. In order to account for differences in chronology length between glacial (shorter) and modern (longer) samples, the following correction (Sokal & Rohlf, 1995) was applied to CV:

$$CV_{corr} = \left(1 + \frac{1}{4n}\right)CV$$

Correlation of Modern c_i/c_a with Climate

To determine correlations of modern c_i/c_a values with climate, monthly temperature and precipitation data were obtained for each site from PRISM (Daly *et al.*, 2008). The PRISM model is ideal for this comparison as it accurately reflects climatic conditions in mountainous

coastal regions with large elevational gradients and complex topography (Daly *et al.*, 2008). Measures of temperature and precipitation alone provided only weak correlations with c_i/c_a, so vapor pressure deficit (VPD) was used for this correlation. VPD is a more integrative climatic parameter that combines water and temperature relationships and is closely linked to evapotranspiration, making this measure more directly related to plant physiology than temperature or precipitation alone. VPD was calculated from monthly average maximum (T_{max}), minimum (T_{min}) and dewpoint (T_{dew}) temperatures using:

$$VPD = \frac{e_s(T_{\max}) + e_s(T_{\min})}{2} - e_s(T_{dew})$$

where $e_s(T)$ is the saturation vapor pressure at temperature T, calculated as

$$e_s(T) = 0.6112 \exp\left(\frac{17.67T}{T + 243.5}\right)$$

In order to correlate ring isotopic composition with VPD, rings of modern trees were associated with specific calendar years**.** Ring width patterns from trees within the same site were correlated and aligned using marker years of high precipitation and growth.

Results/Discussion

The c_i/c_a ratio is driven by two fundamental processes: stomatal conductance, which controls the rate of CO_2 diffusion from the atmosphere into the inter-cellular spaces of leaves; and chloroplast demand for CO_2, which is determined by internal CO_2 diffusion rates to carboxylation sites and photosynthetic biochemistry. Long-term trends in c_i/c_a over evolutionary time scales reflect the degree of coordination between processes affecting CO_2 supply and

demand within the leaf. In addition, shorter-term trends in c_i/c_a (e.g., annual rings) reflect integrated shifts in tree physiology in response to changing environmental conditions within the lifespan of a single individual.

We found that mean c_i/c_a of *Juniperus* was similar between glacial and modern trees (Fig 1.1a; glacial average: 0.52 ± 0.02; modern average: 0.53 ± 0.05; $p > 0.2$;). One possible explanation for this, although one not supported by the literature, is that both stomatal conductance and chloroplast demand for CO_2 remained constant across this expansive period of $[CO_2]$ and climatic change. On the other hand, if only one of these factors predominantly changed through time, there would have been shifts in c_i/c_a, which were not observed here. It is therefore most likely that both stomatal conductance and chloroplast demand for CO_2 were higher during the last glacial period, which would have enhanced CO_2 uptake under limiting carbon conditions. When supply and internal demand for CO_2 covary in the same direction, as has been observed even in highly disparate taxa (Franks & Beerling, 2009a), there are opposing effects on c_i/c_a, likely producing the stabilization effect observed here. When moving into the interglacial period, both stomatal conductance and chloroplast CO_2 demand likely decreased, with the effect of saving water and nitrogen as CO_2 became less limiting. In support of this idea, Ehleringer & Cerling (1995) hypothesized that c_i/c_a represents a metabolic set point that is maintained within species across time. In addition, increases in stomatal conductance are almost always observed in modern C_3 plants grown at low $[CO_2]$ (Gerhart & Ward, 2010), and studies with glacial leaves show evidence for increased stomatal density and decreased stomatal size, which would have increased maximum stomatal conductance in the past (Beerling *et al.*, 1993; Franks & Beerling, 2009b; but also see Malone *et al.*, 1993 for responses of modern plants grown at low $[CO_2]$). The wetter conditions of the last glacial period may have also provided

increased nitrogen availability to support higher leaf nitrogen contents, which may have enhanced photosynthetic capacity.

Despite any physiological adjustments, c_i values remained extremely low in glacial trees relative to modern trees as a result of consistently low c_a throughout the last glacial period (Fig 1.1b; glacial average: 106 ± 6; modern average: 168 ± 20; $p < 0.0001$). Past studies have reported similarly low c_i levels in glacial needles of *Pinus flexilis* preserved in packrat middens (Van de Water *et al.*, 1993; Beerling, 1994). When considering all available rings, the vast majority of glacial c_i values fell outside the range of modern values. In fact, no modern trees experienced c_i values below 114 ppm, and no glacial trees experienced values higher than 120 ppm, leaving only a narrow overlapping range. It is also interesting to note that c_i values of glacial trees never fell below 90 ppm over an integrated annual period. This suggests this may be a limiting concentration below which juniper trees may not maintain a positive carbon budget for basic physiological functions for survival (Campbell *et al.*, 2005).

Inter-annual variation in c_i/c_a, represented by CV_{corr}, was significantly lower in glacial versus modern trees ($p < 0.0002$; Fig. 1.2). More specifically, low and high elevation modern trees showed CV_{corr} values of 8±2% and 8±3%, respectively. The only available Holocene specimen (14.5 kyr BP) showed an intermediate CV_{corr} value of 5%, while glacial specimens showed the lowest levels averaging 3±1%. Furthermore, although modern trees show occasional, short-term periods of low inter-annual variation, these periods are rare. Glacial trees show consistently low variation in c_i/c_a in all cases. In fact, the two oldest glacial *Juniperus* samples (45.1 and 47.6 kya) correspond to the timing of D-O events recorded in Greenland glaciers (Blunier & Brook, 2001). The maintenance of low interannual variation in c_i/c_a, even during time periods of rapid and drastic environmental change that are characteristic of D-O cycles, suggests

that the maintenance of low variation in glacial *Juniperus* physiology was consistent throughout the last glacial period.

In a plethora of past studies, modern *Juniperus* in southern California and the southwestern U.S. exhibits high inter-annual variation in c_i/c_a, mainly as a result of changes in soil water availability from year-to-year (Leavitt & Long, 1989; Feng & Epstein, 1995; Moore *et al.*, 1999; Leffler *et al.*, 2002). In our study, the c_i/c_a of modern trees showed the strongest correlations with monthly or seasonal vapor pressure deficit (VPD; R^2=0.06-0.25; $p<0.05$-0.0001), whereby the months showing the strongest correlations were offset between elevations. Although these correlations were relatively low, similar correlation levels have been reported for modern *Juniperus* in other studies (Leavitt & Long, 1989), and in all cases, soil water parameters and/or VPD correlate most closely with tree physiology (Leavitt & Long, 1989; Feng & Epstein, 1995; Moore *et al.*, 1999; Leffler *et al.*, 2002).

Since glacial climate was much more variable than in modern times, one would expect glacial c_i/c_a to also show higher variation if trees were responding to similar climatic factors. To the contrary, we found reduced levels of inter-annual variation in c_i/c_a during the last glacial period (Fig. 1.2), indicating that a stable environmental factor dominated tree physiology. During the last glacial, [CO_2] was extremely stable from year-to-year (EPICA, 2004), while water availability and temperature were predicted to have been highly variable (Behl & Kennett, 1996; Heusser, 1998; Hendy & Kennett, 1999; Hendy *et al.*, 2002). In our study, extremely low c_i values coupled with reduced variation in c_i/c_a, even under a highly fluctuating glacial climate, point strongly to low [CO_2] constraints on tree physiology. While short-term studies with modern plants grown at glacial [CO_2] show major carbon limitations on physiology, our findings highlight the strength and consistency of low CO_2 constraints over evolutionary time scales.

In conclusion, this study has demonstrated that mean c_i/c_a has been maintained in *Juniperus* between the last glacial period and modern times, despite changes in temperature, precipitation and $[CO_2]$; that glacial c_i levels were extremely low on an annual basis and occur only rarely in modern trees; that a limiting level for *Juniperus* physiology may exist at or near 90 ppm; and that inter-annual variation in c_i/c_a was greatly reduced in glacial *Juniperus*, likely as a result of the constraints of low $[CO_2]$ that over-rode responses to other climatic factors. This is the first direct evidence from trees that actually lived and evolved under low $[CO_2]$ that carbon limitation persisted on an annual basis during the last glacial period. Moreover, our results suggest that the environmental factors that dominate carbon-uptake physiology can vary across geologic time scales, resulting in major alterations in physiological response patterns through time.

Chapter 1 Tables and Figures

Table 1.1. Climate data for glacial La Brea (Heusser, 1998) and modern sampling sites (Daly *et al.*, 2008). Modern samples were collected from San Bernardino National Forest (SBNF) and Angeles National Forest (ANF).

Site category	Site name and coordinates	Elevation (m)	Mean annual precipitation (mm)	Mean annual temperature (°C)
Glacial	La Brea Tar Pits	80	*c.* 600	*c.* 7.5–9.5
	+ 34°3′48″, − 118°21′22″			
Modern high elevation (SBNF)	Big Bear Lake	2830	696	7.3
	+ 34°16′12″, − 116°55′29″			
	Hwy 38 Bend	2300	452	11.1
	+ 34°11′35″, − 116°47′6″			
	Wildhorse Springs	2300	572	8.7
	+ 34°9′52″, − 116°43′14″			
Modern low elevation (ANF)	Mt. Emma Rd	1340	242	14.7
	+ 34°28′55″, − 118°4′2″			
	Littlerock Reservoir	1045	230	14.9
	+ 34°29′42″, − 118°1′36″			
	Lyttle Creek	630	390	18.2
	+ 34°11′22″, − 117°26′11″			

Climate data for glacial La Brea (Heusser, 1998), and modern sampling sites (Daly *et al.*, 2008). Modern samples were collected from San Bernardino National Forest (SBNF) and Angeles National Forest (ANF).

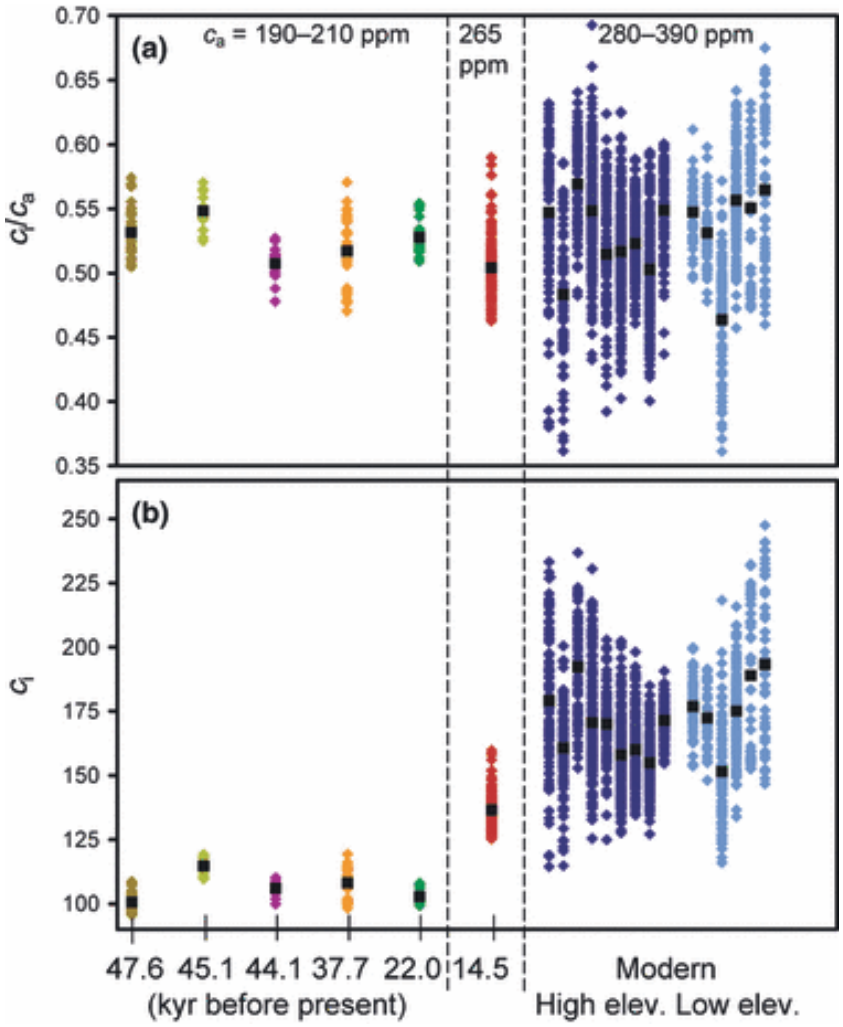

Figure 1.1. Results of stable carbon isotope measurements for glacial and modern *Juniperus* tree rings. (a) intercellular : atmospheric [CO_2] ratio (c_i/c_a values); (b) corresponding intercellular [CO_2] (c_i values). Each point represents an individual tree ring, and vertical groups represent results from all available tree rings for an individual tree, with values stacked from highest to lowest. Glacial samples are shown in different colors, while modern samples are grouped by elevation (to distinguish two different control groups), with means labeled as black boxes. Atmospheric [CO_2] values (c_a) are provided for each group. The range provided for modern samples reflects a temporal gradient experienced by each tree over its lifetime as a result of rapid change sin atmospheric [CO_2] in the modern period.

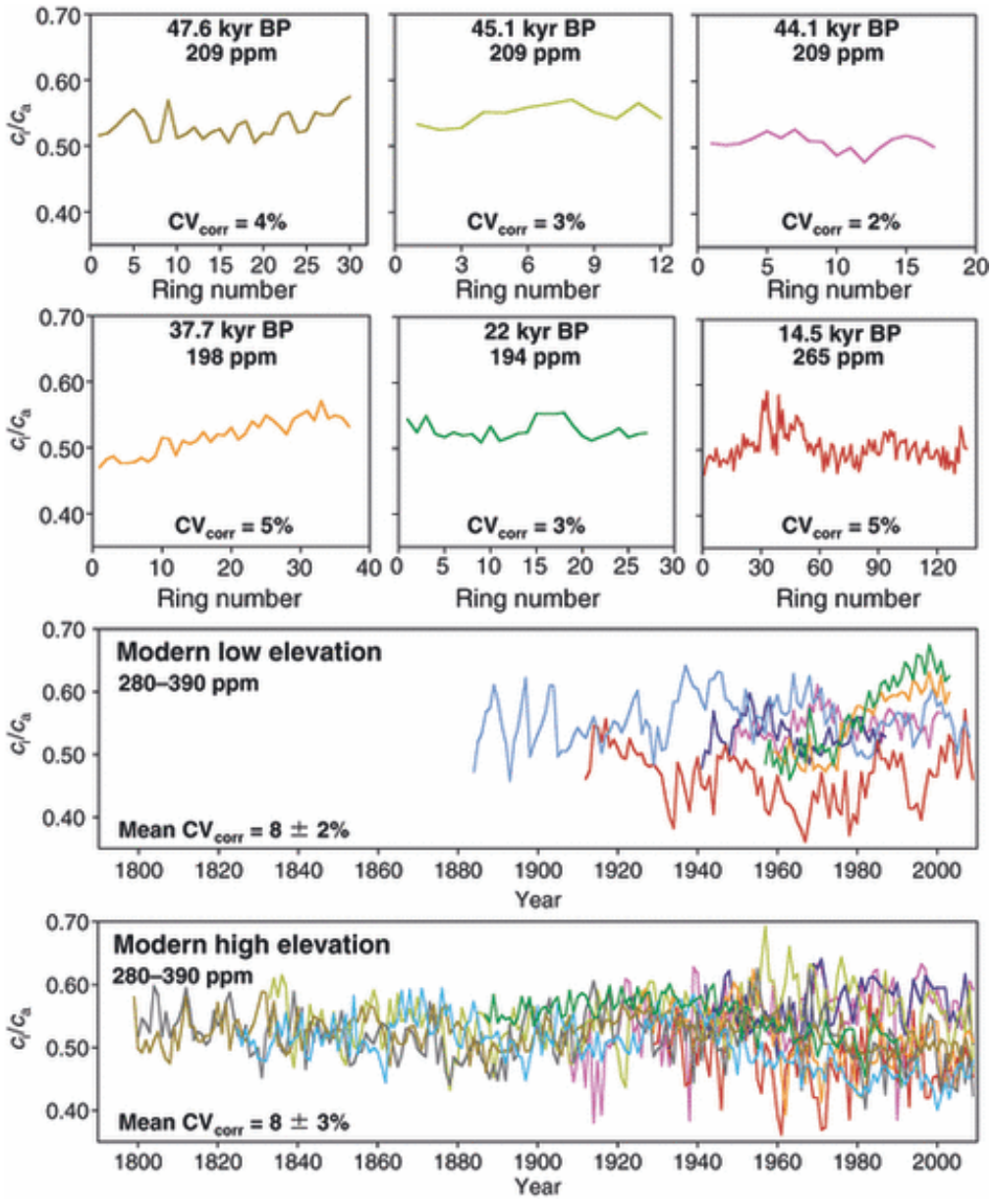

Figure 1.2. Annual responses of intercellular : atmospheric [CO_2] ratio (c_i/c_a) or modern and glacial *Juniperus*. These are the same data as fin Fig. 1, although in this case, data are placed in chronological order throughout the development of each tree. Full chronologies are not available for glacial trees, and therefore data are arranged from youngest (ring number 1) to oldest. Glacial samples are shown in the same colors as in Fig. 1, although

modern samples are given different colors in order to distinguish their responses. ^{14}C age (thousands of years before present, kyr BP), atmospheric [CO_2], and CV_{corr} (interannual variation in c_i/c_a, see the 'Materials and Methods' section for details) are provided for each sample and/or control group for the sake of comparison. The atmospheric [CO_2] range provided for modern samples reflects a temporal gradient experienced by each tree over its lifetime as a result of rapid changes in atmospheric [CO_2] in the modern period.

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Chapter 2: Glacial Trees Maintain Similar Annual Ring Growth as Modern Trees Despite Lower CO_2 Availability

Abstract

Increases in atmospheric [CO_2] since the beginning of the Industrial Revolution have resulted in a current level that is approaching 400 ppm and represents novel conditions for modern plants relative to their glacial counterparts. Glacial plants experienced [CO_2] ranging between 180 and 270 ppm coinciding with glacial-interglacial cycles of the last ~1 million years. Studies of modern plants grown under glacial [CO_2] show strong responses of reduced photosynthetic rates and biomass; however, our understanding of the responses of actual glacial plants remains limited. Investigations of long-term plant responses to changes in atmospheric [CO_2] provide important information on plant physiological patterns over geologic time scales as well as insight into past ecosystem-level responses such as primary productivity and terrestrial carbon storage. Using stable carbon isotope analysis of tree rings, we measured c_i/c_a (internal [CO_2]/atmospheric [CO_2]), c_i, and annual tree ring growth patterns of glacial and modern tree species. These include *Juniperus sp.* preserved within the Rancho La Brea tar pits in southern California and *Agathis australis* from peat bogs in the North Island, New Zealand. We found that both species exhibited constant c_i/c_a since the last glacial period, resulting in severely reduced c_i levels in glacial trees. Though reduced c_i would suggest reduced growth capabilities, glacial *Juniperus* and *Agathis* trees showed no significant difference in mean or maximum ring width compared to modern trees. Additionally, growth-c_i relationships were non-significant for the majority of trees, indicating that increased c_i did not result in increased annual growth, even in glacial conditions of severely reduced [CO_2]. We attribute this lack of along-term CO_2

fertilization of ring growth to possible environmental constraints specific to each region, as well as potential adaptive responses resulting from 10-14 million years of low CO_2 conditions.

Introduction

Temporal changes in atmospheric CO_2 concentrations ($[CO_2]$) over geologic time scales have greatly affected carbon availability to plants. Little is known, however, about how plant physiology and growth responded to conditions of the past, including the last glacial period. For this type of work, most studies have focused on modern plants grown at glacial conditions, whereas fewer studies involve actual glacial plants. The most recent glacial period began approximately 110,000 years ago and reached a maximum for global ice volume 20,000 years ago (Last Glacial Maximum, LGM). During this period, atmospheric $[CO_2]$ reached minimum values of 180-200 ppm and gradually increased to 270 ppm just prior to the Industrial Revolution. Since this time, atmospheric $[CO_2]$ has continued to increase due to fossil fuel combustion, reaching a current value near 400 ppm. The modern $[CO_2]$ experienced by plants is anomalously high, at levels 130-220 ppm higher than what plants have generally experienced over the last 700,000 years (Lüthi *et al.* 2008). Furthermore, atmospheric $[CO_2]$ may not have been this high since the Middle Miocene, 10-14 million years ago (Tripati *et al.* 2009). Because CO_2 is the substrate for photosynthesis, the combination of anomalously high levels of $[CO_2]$ as well as rapid increases in $[CO_2]$ in the modern period has the potential to severely impact plant-atmosphere interactions at the plant physiological through ecosystem levels. It is necessary to anchor plant responses to increasing $[CO_2]$ to plant experiences and adaptations to past conditions of low $[CO_2]$ in order to better understand the full acclimation and adaptation trajectory of plant responses to changing CO_2 availability.

Studies on modern C_3 plants grown at conditions characteristic of the LGM indicate significant physiological ramifications of low [CO_2] (for a review of these effects, see Gerhart & Ward 2010). Under low [CO_2], numerous plant species exhibit 40-70% lower biomass production (Polley *et al* 1993; Sage & Coleman, 2001), 20-30% lower survival rates (Ward & Kelly, 2004), reduced stress tolerance (Sage & Cowling, 1999), and in some instances failure to reproduce (Dippery *et al.* 1995). These results suggest severe impacts of glacial [CO_2], but primarily represent a short-term response within a single generation, as opposed to the effects of long-term adaptation to low [CO_2].

Analyses of glacial plants are often limited by availability of specimens and by a lack of controlled environmental conditions outside of the variable(s) in question (e.g. CO_2); however, these studies have an advantage over studies of modern plants in that they examine plants that lived during a period of sustained low CO_2 conditions over millions of years involving potential adaptive and genetic changes, making these specimens more accurate representations of plant functioning under glacial environments. In this study, we used both regional (sediment cores) and global (ice cores) climate reconstructions to estimate the environmental changes through time, and included modern plant specimens for comparison to glacial samples. This design combines the benefits of analyzing glacial plants while minimizing the impacts of unknown environmental factors.

Studies examining growth effects to [CO_2] above the current level show no consistent response across species or growing conditions. No significant change in growth (e.g. Kilpeläinen *et al.* 2003, Körner *et al* 2005, Dawes *et al.* 2011) is reported just as often as growth enhancements (e.g. Kilpeläinen *et al.* 2005, Dawes *et al.* 2011) and in one study, trees exhibited decreased growth under high [CO_2] (Silva *et al.* 2010). An increase in [CO_2] below the modern

value often has a stronger physiological impact than the same absolute increase in [CO_2] above the modern level (Gerhart & Ward, 2010). Consequently, a lack of consistent growth enhancement in trees to future [CO_2] levels would not preclude the possibility of growth enhancements from glacial to modern [CO_2]. It could be speculated that much of the growth response to increasing CO_2 has already been exploited, resulting in reduced sensitivity to increasing [CO_2] above the modern level. Analysis of long-term growth patterns spanning geologic time scales and wide variations in CO_2 availability (as is done in this study), will help untangle the CO_2-growth relationship through time. Growth enhancements resulting from elevated [CO_2] are often strongest in the first year and decrease in subsequent years (e.g. Körner 2000, Norby *et al* 2000), leading some to question if such responses are caused by subjecting trees to a step increase in [CO_2] as opposed to the gradual increase experienced in nature (Körner 2000, Norby *et al* 2000). Responses are also variable between species (e.g. Dawes *et al.* 2011, Liu *et al.* 2011) and under different environmental conditions (e.g. Kilpeläinen *et al.* 2003, Kilpeläinen *et al.* 2005, Lui *et al.* 2011). To our knowledge, no growth comparisons of increasing [CO_2] on glacial plants have been reported in the literature. Also absent from the literature are growth responses of modern trees subjected to low [CO_2] conditions of the last glacial period, as such studies are primarily performed on herbaceous annuals grown in growth chambers. Consequently, we currently lack data on growth responses to increasing [CO_2] since the last glacial period.

Here, we compare physiology and growth patterns of modern and glacial trees preserved in the La Brea tar pits in southern California, USA (*Juniperus sp*) and peat bogs in the North Island, New Zealand (*Agathis australis*). By combining carbon isotope and ring width analyses, we examined both physiological and growth responses of two tree species to atmospheric [CO_2]

increases since the last glacial period. Our use of samples from the northern and southern hemispheres also allowed us to compare the responses of two coniferous species from regions that experienced different environmental changes (other than [CO_2]) since the LGM. Low [CO_2] impacts on physiology, namely reduced internal CO_2 availability (termed c_i), have been predicted to severely reduce growth capabilities in glacial trees, compared to their modern counterparts; however, the data presented here indicate that glacial trees maintained mean and maximum growth levels similar to that of modern trees.

Materials/Methods

Juniperus Sample and Site Description

Glacial *Juniperus sp.* tree specimens from the Rancho La Brea tar pits (Los Angeles, CA) were collected and ^{14}C dated to a range of time periods between 22 and 47.6 kyr BP. Though specimens from Rancho La Brea cannot be identified to species, wood anatomy characteristics (Ward *et al.* 2005) and modern species distributions with similar climatic conditions suggest they are either *J. californica* or *J. occidentalis* (Vasek 1966). *Juniperus californica* is a shrub-like tree, generally growing to ~8m in height and found on dry, rocky slopes and flats at 750-1600 m in elevation throughout the arid southwest of the United States (Adams, 1993a). *Juniperus occidentalis* is a single-stemmed tree, growing 20-30 m in height, and found on dry slopes and flats at 1,000-3,500m in elevation throughout the western coast of the United States (Adams, 1993b). *Juniperus occidentalis* is capable of living over 1,000, though many old-growth stands are 200-400 years old (Dealy, 1990). Both species are highly drought tolerant, occurring in regions with mild, moist winters, and hot, dry summers. Where the two species overlap, such as in southern California, *J. occidentalis* is generally found at higher elevations than *J. californica*.

Modern *Juniperus* samples were obtained by coring trees currently growing in natural areas in well-drained, non-irrigated soils in the Los Angeles Basin. *Juniperus californica* cores were collected from three low elevation sites in the Angeles National Forest: Mt. Emma Road (2 specimens, N34° 28' 55", W 118° 4' 2"), Little Rock Reservoir (2 specimens, N 34° 29' 42", W 118° 1' 36"), and Lyttle Creek (2 specimens, N 34° 11' 22", W 117° 26' 11"). *Juniperus occidentalis* cores were collected from three high elevation sites in the San Bernardino National Forest: Big Bear Lake (3 specimens, N 34° 16' 12", W 116° 55' 29"), Highway 38 Bend (3 specimens, N 34° 11' 35", W 116° 47' 6"), and Wildhorse Springs (3 specimens, N 34° 9' 52", W 116° 43' 14").

During the last glacial period trees in the vicinity of the La Brea tar pits experienced mean annual temperatures ranging between 7.5 and 9.5°C and mean annual precipitation levels of ~600 mm (Huesser, 1998). Modern low elevation trees sampled in this study experienced higher [CO_2] (280-390 ppm), increased temperature (14.7-18.2°C) and decreased precipitation (240-390 mm) relative to glacial trees. Modern high elevation trees experienced similar temperature (7.3-11.1°C) and precipitation (452-696 mm) conditions to glacial La Brea, allowing for the isolation of the effects of CO_2.

Agathis australis Sample and Site Description

Agathis australis (kauri) is an emergent canopy conifer endemic to New Zealand, that can reach over 30 m in height, 5 m in basal diameter, and 2,000 years in age (Palmer 2006). Though extensive *Agathis*-dominated forests once spanned North Island, widespread logging has left only isolated stands restricted primarily to steep south-facing slopes of spurs and ridges (Newnham 1999). *Agathis* is most competitive under warm, dry growing season conditions

(Buckley *et al* 2000) and has historically alternated in dominance with *Northofagus truncata* (hard beech) in response to precipitation and temperature oscillations of glacial-interglacial cycles (Newnham 1999).

Glacial *Agathis* trees from the Northland region in the North Island, New Zealand were ^{14}C dated to 52.2 to >52.8 kyr BP. These specimens were excavated from peat bogs surrounding Lake Ngatu near Awanui in Northland (8 specimens, S 35° 01' 55", E 173° 11' 52"). Six of the glacial samples were older than the limits of ^{14}C dating, while two dated to 52.2-52.6 kyr BP. Samples were all in close proximity within the bog, and are all likely from this same approximate time range, though a few are pushing the limits of ^{14}C dating. Palynological studies of the marine oxygen isotope stage (IOS) 3b indcate that all glacial samples are from 43-59 kyr BP, when extensive conifer-hardwood forests in Northland were dominated by *Agathis* (Wright *et al.* 1995, Newnham 1999, Elliot *et al*. 2005). Significant ocean cooling following this time period resulted in a shift in terrestrial community structure away from conifer-hardwood forests in favor of grassland and shrub vegetation (Wright *et al.* 1995), supporting similar ages for these tree specimens.

Living *Agathis* trees are federally protected (even from coring), and therefore modern samples were obtained by salvaging *Agathis* remnants from construction sites at which material from older buildings and piers is reclaimed following their dissolution. Consequently, our modern specimens ranged in age from 0.9 to 3.7 kyr BP. Though these specimens represent a wider time window, we refer to them as 'modern' to differentiate them from the much older glacial samples. Modern samples were obtained from four locations in Northland: Lake Ngatu (3 samples), Hick's Road (3 samples, S 35° 17' 7", E 173° 20' 10"), Proctor Road (1 sample, S 35° 20' 19", E 173° 15' 5"), and Herekino Harbour (1 sample, S 35° 17' 3", E 173° 10' 2").

Current environmental conditions at Kaitaia in Northland (centrally located between the modern sample locations) reflect an annual temperature range of 4.6-25.3° C and mean annual rainfall of ~1000 mm year^{-1} (New Zealand Meteorological Service, 2012). Detailed habitat characteristics of *Agathis* trees are unavailable, as samples were not collected from their original growing location. Consequently, mean conditions over a broad range in this region are used here. Palynological and sedimentological studies suggest that Northland was both warmer and wetter during IOS 3b compared with conditions of the region today (Elliot *et al.* 2005, Horrocks *et al.* 2007, D'Costa *et al.* 2008). Specific growing conditions of modern trees are not fully known, and likely do not coincide with glacial conditions meaning the *Agathis* system does not have a tight environmental control (as the *Juniperus* system does with the inclusion of high elevation trees). Instead of a specific environmental control, modern *Agathis* were sampled from a variety of locations spanning modern growing conditions.

Stable Isotope Measurement

Stable carbon isotope ratios were measured on alpha-cellulose from individual tree rings of glacial and modern *Juniperus* and *Agathis*. For each ring, alpha-cellulose was extracted from the full annual ring, providing a measure of integrated responses throughout the entire growing season. Individual rings were separated under a dissecting microscope and alpha-cellulose was extracted using the method described in Ward *et al.* (2005). Previous research has demonstrated the effectiveness of this method in both stable isotope and radiocarbon analysis, documenting high purity levels of alpha-cellulose, and no contamination from the preserving matrix for both tar and peat (Ward *et al.* 2005, Hogg *et al.* 2007, Gerhart *et al.* 2012). We are, therefore, confident that diagenetic processes did not influenced the results presented here.

Isotope analysis was performed on 5 glacial *Juniperus*, 15 modern *Juniperus* (9 *J. occidentalis*, 6 *J. californica*), 8 glacial *Agathis*, and 8 modern *Agathis*. Subsequent to isotope analysis, three additional glacial *Juniperus* specimens were obtained (see Ring Width Analysis section for details). For specimens where the center of the trunk was present in the sample, the 10 rings nearest the center were excluded, as juvenile rings often display erratic physiological patterns. Apart from this exception, all available rings in all samples were analyzed. $\delta^{13}C$ values were calculated using:

$\delta = R_{sample} / R_{standard} - 1$

where R is the ratio of $^{13}C/^{12}C$, using belemnite carbonate from the Pee Dee Formation, Hemingway, SC (PDB) as the standard. Data were converted to "per mil" (‰) notation by multiplying δ values by 1000. For *Juniperus*, $\delta^{13}C_{cell}$ was converted to $\delta^{13}C_{leaf}$ using a constant offset of –3.2‰ that is specific to *Juniperus* (Leavitt & Long, 1982; Ward *et al.*, 2005). For *Agathis*, Δ_{cell} was converted to Δ_{leaf} using a constant offset of 3.6‰ that is specific to *Agathis* (Stephens *et al.* 1999). Carbon isotope discrimination was calculated as:

$$\Delta = \frac{\delta^{13}C_{air} - \delta^{13}C_{leaf}}{1 + \delta^{13}C_{leaf}}$$

Conversion to carbon discrimination is necessary in this case since it accounts for changes in the isotopic signature of the atmosphere through time. $\delta^{13}C_{air}$ was -0.0066 (-6.6‰) during glacial times, and has decreased in modern times to -0.008 (-8.0‰; Leuenberger *et al.*, 1992). From Δ, c_i/c_a was calculated as

$$\frac{c_i}{c_a} = \frac{\Delta - a}{b - a}$$

where a is the fractionation against ^{13}C due to slower diffusion across the stomata (4.4‰) and b is the fractionation against ^{13}C due to Rubisco (27‰).

For each ring, c_i was calculated from the c_i/c_a ratio using c_a values. For modern trees, c_a values were obtained from direct atmospheric measurements (Keeling *et al.*, 2009) and the Taylor Law Dome ice core (Etheridge *et al.*, 1996). For glacial trees, c_a values were obtained from the Vostok and EPICA Dome C ice cores (EPICA, 2004; Lüthi *et al.*, 2008). To obtain the appropriate c_a values, ^{14}C ages of glacial trees were first converted to calendar ages (Beck *et al.*, 2001).

Ring Width Analysis

Ring width was also measured for glacial and modern *Agathis* and *Juniperus* specimens. For *Agathis*, ring width was measured for the same samples from which carbon isotope measurements were obtained (totaling 8 modern and 8 glacial samples). For *Juniperus,* ring width was analyzed for the same modern samples (totaling 15 modern trees) and for five total glacial samples. The five glacial samples included two of the glacial samples for which carbon isotope measurements were taken and three additional trunk specimens which were obtained for the ring width analysis from the same La Brea location, but which were not included in the carbon isotope dataset. Three glacial *Juniperus* samples used in the carbon isotope analysis were excluded from ring width analysis, as they could not be definitively identified as trunk specimens. As with isotope analysis, the 10 rings nearest the center were excluded; otherwise, all available rings were measured.

Samples were imaged at high resolution using a flatbed scanner and from these images, ring widths were measured to the nearest 0.1 mm using Photoshop. We present raw ring width here (as opposed to ring width indices), since the focus of our analysis is physiology and growth comparisons, as opposed to classic dendrochronological approaches where the motive is

understanding relative ring width for the purpose of climate reconstruction (Hättenschwiler *et al* 1997, Rolland *et al* 1998, Telewski *et al* 1999, Kilpeläinen *et al*. 2003, Kilpeläinen *et al.* 2005). Compression of rings during growth can result in variable ring width depending on where in the cross section of the tree the ring is measured. For modern *Juniperus*, we accounted for this problem by selecting only straight trees, which tend to minimize compression. We also cored all modern trees on the south side, as trees from the same location tend to show similar compression patterns based on slope and aspect. For modern *Agathis*, many samples consisted of entire tree cross-sections, from which we could select a measurement section that showed the least compression. For glacial trees, we were unable to fully account for such variation, as we have limited sections of preserved wood, albeit we have a relatively large sample size with high environmental control for paleo-ecological work.

Statistical Analyses

Because ring widths sampled from different years within the same individual tree can exhibit autocorrelation, nested ANOVAs were used to compare c_i/c_a, c_i, and mean raw ring width in both *Juniperus* and *Agathis.* In all cases, tree ID was treated as a random variable, nested within the fixed variable of time period (glacial or modern). For maximum raw ring width comparisons, the single largest value for each tree was pooled by time period and a one-way ANOVA was used to compare glacial and modern means in each species. To compare growth-c_i relationships, trees were first checked for age trends in growth. If an individual tree did not exhibit a significant age trend, ring widths were not standardized; however, if an individual tree did exhibit a significant age trend (either positive or negative), ring widths were standardized by

dividing the raw ring width by a 20-year sliding mean. Linear regression was then performed on growth-c_i relationships for each tree. All statistical analyses were performed using JMP 5.1.

Modern trees of *J. californica* and *J. occidentalis* exhibited statistically identical responses in c_i/c_a, c_i, mean ring width, and maximum ring width. Consequently, the two groups were combined to form one modern *Juniperus* dataset, which was then compared in total to glacial samples.

Results

Here we compare c_i/c_a, c_i, and growth responses between two tree species from different hemispheres between the last glacial period and the present. Modern and glacial c_i/c_a did not significantly vary across time in either species (Fig 2.1a,b). In both species, c_i values were significantly lower during the last glacial period (Fig 2.1c,d). In both *Juniperus* and *Agathis*, modern and glacial c_i values exhibited only a narrow overlapping window of 3-6 ppm, with less than 1% of all rings (glacial and modern) falling in this range. Glacial and modern samples did not significantly differ in mean raw ring width (Fig 2.1e,g), or maximum raw width (Fig 2.1f,h) for either species.

Ten modern *Juniperus*, five glacial *Agathis*, and four modern *Agathis* exhibited significant age growth trends. After standardizing those for which an age-growth trend was present, one glacial *Juniperus* specimen showed a negative growth-c_i trend ($p = 0.07$; Fig. 2.2a) and two modern *Juniperus* showed positive trends ($p < 0.01$; Fig. 2.2b,c). One glacial *Agathis* showed a positive growth-c_i trend ($p < 0.0001$; Fig. 2.2d) and two modern *Agathis* showed negative trends ($p < 0.007$; Fig. 2.2e). All other trees (1 glacial *Juniperus*, 13 modern *Juniperus*, 7 glacial *Agathis*, and 6 modern *Agathis*) exhibited no significant growth-c_i relationships.

Discussion

When growing modern plants under glacial conditions of low [CO_2] severe physiological impacts are observed, including 50% reductions in biomass and net photosynthesis and even failure to reproduce (Gerhart & Ward, 2010). These studies have primarily relied on short-lived herbaceous annuals and so responses of long-lived woody species are still relatively unknown. Studies utilizing preserved glacial plant material address gaps in our knowledge in two ways, by addressing responses of long-lived woody species, and by analyzing the responses of plants that actually lived during the last glacial period.

Here, we analyzed the relationship between atmospheric and internal [CO_2] (c_i/c_a and c_i calculated from carbon isotope values) as well as annual ring width patterns in *Juniperus* and *Agathis* samples dating to the last glacial period with modern controls from the same region. From annual ring width measures, we found that growth was not significantly different between glacial and modern trees despite reduced c_i values in glacial trees. Growth-c_i analysis suggests that glacial trees differed physiologically in their response to c_i. Though the majority of trees displayed no growth-c_i relationship, the few significant relationships exhibited shifts in the direction of the relationship between time periods (e.g. negative glacial *Juniperus* trends, but positive modern *Juniperus* trends). Below, we discuss the possible mechanisms behind these findings and the implications of our data for ecosystem-level processes.

Both *Juniperus* and *Agathis* showed constant c_i/c_a values throughout the last 50,000 years. Constant c_i/c_a likely resulted from decreases in both stomatal conductance and photosynthetic capacity as [CO_2] increased through time. A decrease in stomatal conductance would lower the c_i/c_a ratio by allowing less CO_2 into the leaf, while a decrease in photosynthetic capacity would raise the c_i/c_a ratio by slowing the uptake of CO_2 inside the leaf. Concurrent

decreases in both measures in response to rising atmospheric [CO_2] then result in a constant c_i/c_a ratio (Ehleringer & Cerling 1995). It is possible that stomatal conductance and photosynthetic capacity show no response to increasing [CO_2], and a lack of change in both parameters results in constant c_i/c_a; however, increases in both parameters at low [CO_2] are well- documented in a wide variety of taxa (Franks & Beerling 2009). Constancy in the c_i/c_a ratio has been demonstrated in other studies (Feng 1998, Ward *et al* 2005, Gerhart *et al* 2012), and was predicted theoretically by Ehleringer & Cerling (1995). It is worth noting, however, that constant carbon discrimination (and therefore, c_i/c_a) through time is not exhibited by all species or under all conditions (Beerling *et al* 1993, Polley *et al* 1993, Van de Water *et al* 1994, Feng & Epstein 1995, Beerling 1996) In these cases, short-term changes in [CO_2] can shift c_i/c_a in herbaceous species (Polley *et al* 1993) and, even in trees c_i/c_a responses have been especially dynamic over the last two centuries, due to rapid and drastic changes in atmospheric conditions during this time (Feng & Epstein 1995, Beerling 1996, Feng 1998).

Constant c_i/c_a, coupled with reduced atmospheric [CO_2] in glacial periods, resulted in drastically reduced c_i values for glacial trees. For both *Juniperus* and *Agathis*, glacial c_i values were almost entirely below the range of modern values on an annual basis, and reduced on average across the life of the tree by 50-60 ppm. Drastically reduced internal CO_2 availability would suggest significantly reduced growth capabilities in glacial trees (Ward *et al* 2005, Gerhart *et al* 2012), though such a reduction was not observed in this study.

Contrary to this hypothesis, both *Juniperus* and *Agathis* showed statistically identical mean raw ring width between glacial and modern samples, despite a significant increase in atmospheric [CO_2] levels from the last glacial period to today that coincided with increases in internal CO_2 availability (c_i). In addition, neither species showed a significant difference between

glacial and modern maximum ring width, indicating that even in years of ideal environmental conditions, increased [CO_2] did not result in increased growth.

The majority of trees (modern and glacial of both *Juniperus* and *Agathis*) exhibited no significant relationship between annual c_i and growth (Fig. 2.2), indicating that differences in internal CO_2 availability did not translate into differential growth, even over the length of an individual lifespan. Modern annual plants exhibit strong positive growth responses to increasing c_i at [CO_2] below the modern value (Sage, 1994). Long-lived tree species, however, can show strongly differing growth responses to increasing [CO_2] (e.g. Körner *et al* 2005, Dawes *et al.* 2011). From the data shown here, it appears that glacial [CO_2] was not limiting to growth for either *Juniperus* or *Agathis*. What is more surprising is that even on the scale of a single lifetime, the majority of glacial trees show no significant growth enhancement to increasing c_i, despite the vast reduction in c_i levels experienced by glacial trees. This lack of significant response in glacial trees may be the result of adaptation or acclimation to low [CO_2]. For example, increases in stomatal conductance and photosynthetic capacity could have allowed glacial trees to increase carbon uptake for growth despite low atmospheric CO_2 availability. Increases in photosynthetic capacity would have required increased investment of resources by the plant in photosynthetic machinery (such as Rubisco), which could have been advantageous at the time despite the increased cost.

Interestingly, the few trees that exhibit significant growth-c_i relationships display differing response patterns across time (from glacial to modern) and between species. Specifically, significant trends in *Juniperus* shifted from negative to positive from glacial to modern times (Fig. 2.2a, n=1; Fig. 2.2b n=2); and significant trends in *Agathis* shifted from positive to negative (Fig 2.2d, n=1; Fig. 2.2e, n=2). Admittedly, our sample size is by necessity

low, however, these trends suggest important differences in *Juniperus* and *Agathis* responses to changing atmospheric and environmental parameters through time. For *Agathis*, the shift from positive (glacial) to negative (modern) growth-c_i trends may result from increasing aridity across this time period, since drier conditions generally result in reduced stomatal conductance in order to avoid xylem embolism (e.g. Héroult *et al.* 2012), resulting in a negative growth-c_i relationship in modern *Agathis* due to photosynthetic drawing down of internal CO_2. The shift from negative (glacial) to positive (modern) growth-c_i trends in *Juniperus* is unexpected, as this region also experienced increasing aridity from glacial to modern times. Positive growth-c_i trends are only exhibited by higher elevation modern *Juniperus*, which may be the result of cooler and wetter conditions at these sites compared to other modern *Juniperus* sites, allowing for less conservative stomatal behavior. The negative growth-c_i trend exhibited by the glacial *Juniperus* sample is curious, but may be an isolated response to microclimate conditions. Negative growth-c_i trends may result when photosynthetic capacity increases more quickly than stomatal conductance, meaning internal CO_2 is drawn down faster than it can be replenished from the atmosphere. If this glacial *Juniperus* experienced unusually dry microclimate conditions (which would reduce stomatal conductance), high photosynthetic capacity could produce a negative growth-c_i trend. The counter-intuitive trend between glacial and modern *Juniperus* may be a result of the relatively extreme anisohydric hydraulic strategy of this genus, whereby *Juniperus* maintains water uptake even under droughted conditions, resulting in unusually low water potentials and increased xylem cavitation during the growing season (West *et al.* 2007). If additional samples of glacial *Agathis* and *Juniperus* were to become available, these trends could be verified; however, these patterns provide some tantalizing suggestions as to the underlying factors driving identical patterns across time in c_i/c_a, c_i, and mean and maximum growth.

It is important to note that our results reflect only changes (or lack thereof) in radial trunk growth, leaving the potential for alterations in other growth parameters, including trunk height, branch growth, below-ground production, and leaf area that may alter the relationship between radial trunk growth and total growth. Some studies have documented a significant increase in tree height with increasing [CO_2] (Tissue *et al.* 1997, Percy *et al.* 2002, Buitenwerf *et al.* 2012) while others have found no significant effect (Norby *et al.* 2005, Kilpeläinen *et al.* 2005), or variable effects depending on environmental conditions (Messaoud & Chen, 2011). Elevated [CO_2] conditions have also been shown to result in increased branch growth (Tissue *et al.* 1997) and increased allocation to below-ground root growth (Curtis & Wang 1998, Norby *et al* 2004), though mature forests can also show reduced allocation to roots under high [CO_2] (Bader *et al.* 2009). Vegetation models and LGM pollen records both indicate reduced leaf area index (LAI) in response to low [CO_2] conditions (Tissue *et al.* 1997, Williams *et al.* 2008). While these studies do not always give a clear picture of growth effects in response to increases in [CO_2] since the LGM, they highlight conditions in which radial growth increment may not be the only growth parameter affected by increasing [CO_2]. Thus, until we know more about allometric growth patterns between glacial and modern conditions (assuming such studies are even possible), we need to remain cautious in our interpretations of radial growth patterns.

In general, growth enhancement to increasing [CO_2] above the current level is not as high as might be expected, particularly under natural conditions where CO_2 levels interact with other limiting factors (Ainsworth & Long 2005). In some cases, a CO_2 fertilization effect may not be present at all, or may be weaker than growth responses to other climatic parameters (Girardin *et al* 2011) suggesting that modern plants may be limited in translating recent increases in [CO_2] into increased growth due to constraints imposed by drought, elevated temperature, or nutrient

limitations (Sage & Cowling 1999, Polley *et al* 1994, Lewis *et al* 2010). The *Juniperus* and *Agathis* systems reported here experienced different long-term environmental changes which could have potentially different impacts on overall growth patterns. Specifically, *Juniperus* experienced increasing temperature and decreasing precipitation through time (though high elevation trees controlled for this shift) while *Agathis* experienced decreasing temperature and precipitation through time. *Juniperus* is well-adapted to the mild winters and hot, dry summers of southern California, but cooler and wetter conditions during the last glacial period may have reduced drought stress through both increased soil water availability and reduced evaporative water loss. A lack of difference in overall growth patterns in *Juniperus* could be interpreted as a shift in limiting factors from [CO_2] during the last glacial period to water availability in modern times. Our experimental design was constructed to account for these shifting environmental factors (through the inclusion of high elevation trees), but this shift could explain why glacial trees exhibit similar growth even to low elevation trees under warmer and drier growing conditions. Our previous work on *Juniperus* identified differences in interannual variability in c_i/c_a between glacial and modern trees, which we attributed to this same shift in limiting factors, namely over-riding CO_2 controls in glacial periods, shifting to influence of water availability in modern times (Gerhart *et al* 2012). *Agathis* is competitive under drier growing conditions experienced in modern times, yet these environmental conditions coupled with CO_2 increases still fail to produce a significant enhancement in modern *Agathis* growth. It is more difficult to attribute this lack of increase to specific environmental conditions as our *Agathis* system is limited in its controls (i.e. specific growing conditions of modern individuals are not fully known and likely do not coincide with glacial conditions); however, modern *Agathis* were sampled from diverse areas spanning the modern growing conditions to assess the full range of environmental

effects. A lack of growth enhancement in *Agathis* could be due to nutrient limitations in modern growing conditions, as forest remnants are often limited to leached soils on steep ridges and slopes (Newnham 1999). These remnants likely do not represent the full range of microhabitat conditions for *Agathis* prior to deforestation, which is estimated to have begun c. 1300 A.D. (Mildenhall & Orpin 2010). With no deforestation pressure, glacial *Agathis* individuals may have been able to colonize more suitable microhabitats, potentially reducing growth-limiting factors in glacial trees.

In addition to environmental constraints, modern plants may also be constrained by adaptations that were advantageous under low [CO_2] conditions, but which may be maladaptive in a high [CO_2] environment. For example, preferred allocation of carbon to storage as non-structural carbohydrates instead of growth, or excessive investment in enzymes (such as Rubisco) could prevent plants from exploiting the productivity advantage of high [CO_2] (Sage & Coleman 2001). Evolutionary constraints could be especially pronounced in long-lived species, such as *Juniperus* and *Agathis*, which would respond to selective pressures more slowly than plants with shorter life-spans, and therefore may take longer to adapt to modern high [CO_2] conditions. It is also worth noting that increasing [CO_2] does not present a stress for plants, and therefore plants may lack, or delay, acclimation or adaptation strategies to increasing [CO_2] (Makino & May 1999). In fact, photosynthetic mechanism responses to [CO_2] are often attributed to secondary factors (such as starch accumulation or decreased N content) as opposed to direct impacts of high [CO_2] (Makin & May 1999, Sage & Coleman 2001) and may show no response where such secondary factors are reduced (such as in plants with starch storage organs, Makin & May 1999). Responses of physiological mechanisms (such as photosynthetic

acclimation) to such secondary factors as opposed to responding directly to increased [CO_2] may partly explain the variety of growth responses plants exhibit to increasing [CO_2].

Tree responses to changing atmospheric [CO_2] on a long-term, evolutionary scale are also an important factor in ecosystem-level responses to low [CO_2], and very little is known about this issue (but see Ward *et al*. 2000). Global vegetation modeling suggests changes in atmospheric [CO_2] over geologic time scales drove expansion and contraction of forests (Bond *et al.* 2003, Harrison & Prentice 2003, Crucifix 2004), impacted tree response to disturbance such as fire (Bond *et al* 2003), and altered forest canopy characteristics (Cowling *et al.* 2008). Such alterations in forest extent and structure result in cascading effects at the ecosystem level, including altered migration patterns for plant and animal species (Cowling *et al.* 2008), altered ecosystem albedo that can result in warmer and drier conditions (Levis *et al.* 1999), and reduced global NPP and terrestrial carbon storage (François *et al.* 1998, Turcq *et al.* 2002, Otto *et al.* 2002, François *et al.* 2006). It is estimated that, compared to pre-Industrial levels, the LGM exhibited 27-36% lower global gross primary productivity (Prentice *et al* 2011) and 300-700 PgC lower terrestrial carbon storage (Bird *et al* 1994, Bird *et al* 1996, Street-Perrott *et al* 1998, Beerling 1999, Ikeda &Tajika 2003, Köhler & Fischer 2004, Prentice *et al* 2011). Many of these models rely on direct physiological effects of CO_2 on plants, such as reduced growth, which are then scaled up to ecosystem-level impacts (Prentice & Harrison 2009, Prentice *et al* 2011). If these direct effects of CO_2 on long-lived plants are not as consistent or severe as previously thought (as we have shown here), these estimates may be biased towards lower estimates of ecosystem characteristics such as NPP and terrestrial carbon storage.

In conclusion, we have shown here that glacial and modern *Juniperus* and *Agathis* trees exhibit constant c_i/c_a throughout the last 50,000 years, resulting in drastically reduced c_i levels in

glacial trees. Despite the implications that low c_i levels should correspond to decreased growth in glacial trees, glacial *Juniperus* and *Agathis* trees exhibited similar mean and maximum growth patterns relative to their modern counterparts. Growth consistency occurred in the *Juniperus* system, which included direct modern controls accounting for changes in temperature and precipitation since the last glacial period, and in the kauri system, which incorporated modern controls from a wide range of areas with large environmental differences. Interestingly, we see that both species, having different life history characteristics, experiencing different environmental changes since the LGM, and occurring in different hemispheres, show identical responses of c_i/c_a, c_i, and ring width to increasing atmospheric [CO_2]. We attribute the lack of CO_2-induced growth enhancement to potentially limiting environmental conditions in modern times, and to potential lingering adaptations to low [CO_2] conditions, both of which may constrain the ability of *Juniperus* and *Agathis* to fully exploit increases in CO_2 availability in modern times. We conclude that significant and sustained growth enhancements in response to past increases in [CO_2] may not have occurred in some species, and such a finding may involve reassessment of levels of primary productivity and terrestrial carbon storage during the last glacial period, particularly since our work involves some of the few actual glacial plant systems in existence.

Chapter 2 Figures

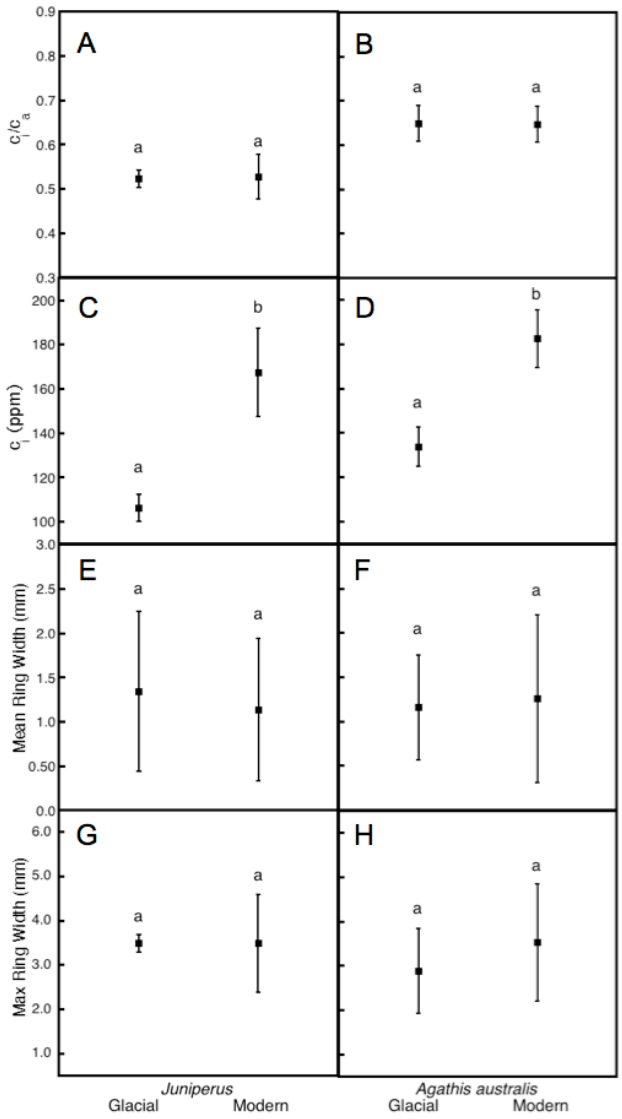

Figure 2.1 Physiological and growth responses of glacial and modern *Juniperus sp.* and *A. australis*. A) *Juniperus* c_i/c_a, B) *A. australis* c_i/c_a, C) *Juniperus* c_i, D) *A. australis* c_i, E) *Juniperus* mean ring width, F) *A. australis* mean ring width, G) *Juniperus* maximum ring width, and H) *A. australis* maximum ring width. Data are shown as group mean with error bars of one standard deviation. Letters above the error bars represent significance, with different letters indicating $p<0.0003$. *Juniperus* data presented in panels A and C are reproduced in summary from Gerhart *et al* 2012.

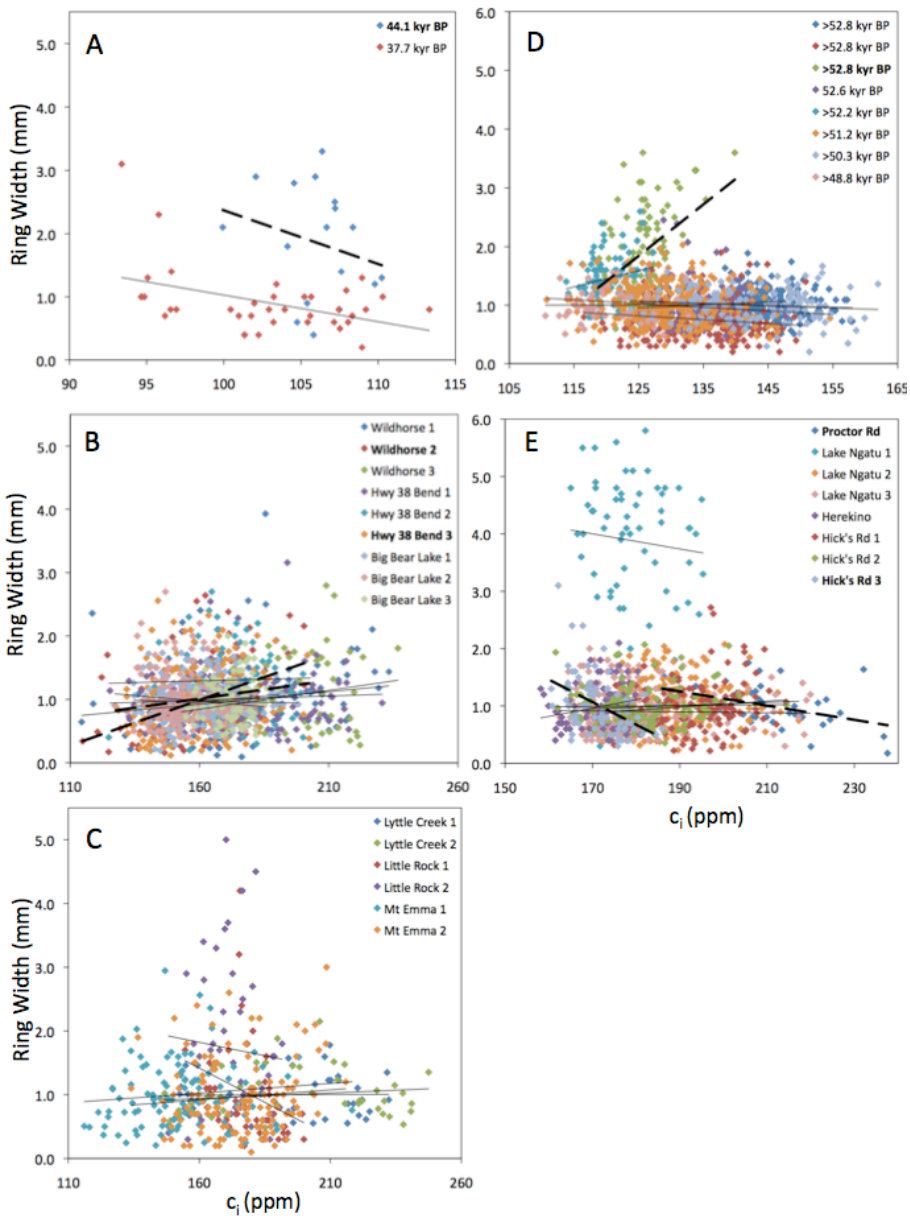

Figure 2.2 Growth-c_i responses of glacial and modern *Juniperus*. A) Glacial *Juniperus*, B) Modern *J. occidentalis,* C) Modern *J. californica*, D) Glacial *Agathis,* and E) Modern *Agathis.* In all panels, solid trend lines are non-significant, while dashed trend lines are significant to $p < 0.01$. Individual trees with significant growth-c_i trends are also bolded in the legend. Modern *Juniperus* are separated into two panels for easier viewing of individual patterns, though these groups showed statistically identical response patterns.

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Chapter 3: El Niño Southern Oscillation and *Juniperus* Physiology

Summary

The El Niño Southern Oscillation (ENSO) is currently the most significant source of global climatic variability. Although direct impacts are felt only in the tropical Pacific, ENSO teleconnections extend across the globe. Currently, knowledge of the sensitivity of ENSO patterns to changes in climatic boundary conditions is limited, and elucidating paleo-ENSO patterns is plagued by uncertainties in model parameters as well as varying results from proxy-based reconstructions. Tree rings provide a unique record of climatic variation given their long chronologies and annual resolution. Some of the strongest ENSO tree-ring signals are found in conifer ring chronologies from the southwestern United States. In these arid regions, ENSO-induced increases in winter temperature and precipitation register as wider annual rings. Oxygen isotopic content of ring alpha-cellulose reflects dynamics of water availability during growth and so can also be used to reconstruct atmospheric conditions. This study applies an ENSO prediction model developed by Nippert *et al.* in 2010 to modern trees at high altitudes that are growing in climates similar to lowland conditions during the last glacial period. Interestingly, we find that *Juniperus* physiological responses to El Niño conditions at higher altitudes greatly differ from those at lowland sites used to develop the Nippert *et al.* (2010) model. Under less water-limiting conditions, *Juniperus* does not respond as strongly or as predictably to El Niño conditions, potentially confounding paleo-proxy ENSO reconstructions in this region.

Introduction

The El Niño Southern Oscillation (ENSO) phenomenon is currently the most significant source of global climatic inter-annual variability (Allan, 2000). Although ENSO directly impacts

the tropical Pacific, indirect effects resulting from altered global oceanic and atmospheric circulation patterns (teleconnections) extend well beyond the tropics (Hoerling & Kumar, 2000). El Niño conditions produce droughts and wildfire throughout Indonesia and southern Africa, and heavy rainfall, floods and mudslides along the western coasts of the Americas (Kovats, 2000). Currently, knowledge of the sensitivity of ENSO patterns to changes in global climatic conditions is extremely limited (Timmerman *et al.*, 1999; Merkel *et al.*, 2010). Though significant progress has been made recently in understanding current ENSO dynamics, behavior of this system under different background climatic states (both past and future) remains enigmatic, as proxy-based reconstructions and coupled ocean-atmosphere models show a wide variety of responses (Merkel *et al.*, 2010). The recent occurrence of anomalously strong El Niño events (in 1982 and 1997) inspired a resurgence of research into ENSO dynamics under past, current, and future climate conditions (Rosenthall & Brocolli, 2004; Cane, 2005).

ENSO activity has a significant effect on southern California, where El Niño conditions generate increased precipitation and temperature during the growing season (Schonher & Nicholson, 1989). Previous dendrochronological research has indicated the strongest El Niño tree-ring signals are found in conifer ring chronologies of northwestern Mexico and the southwestern United States, where warmer and wetter conditions translate to increased growth (i.e. wider ring width; Cook *et al.*, 2000). ENSO chronologies have been developed on several coniferous species from this region, including Douglas fir, ponderosa pine, and Mexican bald cypress (Cook *et al.*, 2000). In these studies, ENSO patterns are elucidated from tree ring chronologies by correlating standardized ring width indices with a climatic measure of El Niño, (such as the southern oscillation index, SOI; or sea surface temperatures, SSTs) or with

anomalous ENSO-induced rainfall patterns (through total precipitation amount or relative measures such as the Palmer Drought Severity Index; Cook *et al.*, 2000).

In addition to growth trends, climate reconstructions are more commonly incorporating oxygen isotopic signatures of plant tissues, such as tree ring cellulose (Ballantyne *et al.*, 2005; Masson-Delmotte *et al.*, 2005; Treydte *et al.* 2006; Loader *et al.,* 2010). Oxygen isotope signatures of cellulose extracted from tree rings reflect numerous environmental parameters, including the water source for the plant (Roden *et al.*, 2000; Danis *et al*, 2006), atmospheric humidity (Roden *et al.*, 2000), temperature (Liu *et al.*, 2009), precipitation (Ferrio & Voltas, 2005), and large-scale atmospheric circulation patterns (Weiguo *et al.*, 2004; Roden & Ehleringer, 2007; Sidorova *et al.*, 2008; Liu *et al.*, 2009). The oxygen isotope signature of leaf water is a function of the isotopic signature of water taken up by the root (i.e. meteoric water). The oxygen isotopic signature of leaf water is then influenced by evaporative enrichment due to transpiration at the leaf surface (where lighter ^{16}O evaporates more quickly than ^{18}O), and biochemical effects during the formation of specific tissue (Roden *et al.*, 2000). For wood cellulose synthesis, these biochemical effects include fractionation during the synthesis of photosynthetic sugars in the leaf, and exchanges of oxygen molecules in these sugars with xylem water in the trunk (which is not evaporatively enriched; Roden *et al.,* 2000) The oxygen isotopic signature of meteoric water is itself impacted by environmental conditions during initial evaporation, cloud condensation temperatures, and preferential fall-out of precipitation containing the heavier isotope as the cloud mass moves across the landscape (i.e. Rayleigh distillation; Roden & Ehleringer, 2007). Consequently, numerous environmental parameters, spanning much longer than just the growing season of the plant, can play important roles in determining the oxygen isotopic signature of tree ring cellulose. Additionally, these

environmental signals can be confounded by species- or individual-specific effects (Roden *et al.*, 2005; Reynolds-Henne *et al.*, 2009), increasing the complexity of the relationship between oxygen isotope signatures and climate. The impacts of these numerous factors on isotopic signatures of plant tissues were modeled in detail by Roden *et al.* (2000), which remains the current standard for understanding environmental effects on plant oxygen and hydrogen isotopic signatures.

In 2010, Nippert *et al.* developed a Baysian ENSO prediction model using modern *Juniperus*-ENSO relationships in southern California, whereby growth and oxygen isotope signatures of tree rings were used to predict El Niño occurrences. The foundation for prediction of an El Niño event relied on the underlying relationship between growth and oxygen isotope signatures in El Niño and non-El Niño years. Specifically, growth and oxygen isotopic signature are positively correlated in El Niño years and negatively correlated in non-El Niño years. The Nippert *et al.* (2010) model expanded on the Roden *et al.* (2000) model by allowing for prediction of ENSO events when detailed environmental data is not available (Nippert *et al.*, 2010). Consequently, this model would be ideal for reconstructing past ENSO patterns using preserved glacial *Juniperus* from the southern California area. Though some work has been done on glacial *Juniperus* physiology from this region (Ward *et al.*, 2005; Gerhart *et al.*, 2012), little is known about the impact of extreme climatic events on glacial trees in this area. In this study, we begin by testing the Nippert *et al* (2010) model against modern *Juniperus* from high elevations in the San Bernardino Mountains in southern California, which more closely simulate glacial *Juniperus* growing conditions. If the model accurately predicts El Niño occurrences at these additional sites, which represent a broader sampling of *Juniperus* growing conditions, we then have validation to apply the model to glacial *Juniperus* samples to assess El Niño frequency, as

well as effects of ENSO on tree physiology, during the last glacial period. If the model does not accurately predict El Niño occurrences at higher elevations in modern *Juniperus*, then there is need to delve deeper into the climate-physiology relationships at these sites to determine how El Niño conditions are differentially experienced under different background environmental states (i.e. higher precipitation and lower temperature).

Materials and Methods

The ENSO Model

The model used in this study is a Bayesian model for identifying El Niño events using a combined analysis of tree ring width and oxygen isotopic composition of ring alpha-cellulose, which was developed using modern *Juniperus* trees in the Los Angeles Basin (Nippert *et al.*, 2010). This model advances the traditional dendrochronological approach by incorporating oxygen isotope analysis, basing El Niño designation on the relationship between growth and isotopic signatures of rings, as opposed to growth alone. Oxygen isotope measurements of tree ring cellulose provide an important proxy for identifying El Niño years because these measurements are driven by precipitation water sources that show altered oxygen isotope patterns based on intensity of storms, as well as cloud condensation temperature that vary seasonally and between El Niño and non-El Niño years in southern California. This model is complementary to previous tree-ring models (Roden *et al.*, 2000) in that it is built on the same principles of oxygen isotopic signatures in wood; however, the Nippert *et al.* model operates independently of direct climatic parameters, allowing us to scale back in time when specific environmental factors are not always known. This approach is unique in that it explicitly accounts for variability in the relationship between climate and tree ring characteristics during El

Niño and non-El Niño years, accommodating uncertainties in model parameters that have plagued traditional methods.

The model developed by Nippert *et al.* (2010) builds on previous physiological models quantifying hydrogen and oxygen isotope ratios of tree-ring cellulose in terms of source water and humidity during tree growth, as well as biochemical and biophysical fractionation factors associated with carbohydrate transport and cellulose synthesis (Roden *et al.*, 2000). The model combines ring width and $\delta^{18}O$ values to predict the likelihood that a given ring was laid down during an El Niño year. Most importantly, this model accounts for differences in the climate-growth relationship that occurs in southern California, whereby ring width and oxygen isotopic signature are positively correlated during El Niño years, but negatively correlated during non-El Niño years (Fig. 3.1). More specifically, for non-El Niño years on the southern California coast, fractionation of oxygen isotopes in rings is driven by the amount of precipitation during the growing season; high growing season precipitation produces wider rings and lighter oxygen isotope signatures (lower $\delta^{18}O$ values). During El Niño events, however, wider tree rings correspond to heavier oxygen isotope signatures (higher $\delta^{18}O$ values), reflecting rainfall of water vapor formed under warmer cloud condensation temperatures when warmer conditions prevail deeper into the growing season. Exploiting this unique pattern, the model identifies the probability that a given tree ring was produced during an El Niño year in this region (Fig 3.2.)

For the study described here, years were categorized as El Niño (La Niña) events based on SST anomalies in the Niño3.4 region (5^oN-5^oS, 120^o-170^oW). Numerous ENSO classification indices exist, though none are universally accepted by the scientific community. Studies have shown that the Niño3.4 index is particularly sensitive to ENSO events (Hanley *et al*, 2003), and therefore more likely to reflect changes in ENSO due to altered climatic conditions (for example,

expansion or contraction of the oceanic cold tongue; Bush *et al.*, 2007). Following the method of Hanley *et al.* (2003), a year was classified as El Niño (La Niña) when the 3-month running mean of the Niño3.4 SST anomalies exceeded 0.5^{o}C (-0.5 ^{o}C) for five or more consecutive months, which must include October-November-December (SST anomalies obtained from CPC, 2011). Between 1969 and 2009 (the time period covered by the modern *Juniperus* samples), this rubric identified 14 El Niño years (1970, 1972, 1976, 1977, 1982, 1986, 1987, 1991, 1994, 1997, 2002, 2004, 2006, and 2009) and 12 La Niña years (1970, 1971, 1973, 1974, 1975, 1984, 1988, 1995, 1998, 1999, 2000, and 2007), leaving 15 neutral years.

Juniperus Sample and Site Description

This research utilizes glacial *Juniperus* material preserved in the Rancho La Brea tar pits in Los Angeles, CA, which date to 14-48 kyr BP (1 Holocene, 5 glacial specimens). Though species-level identification is not possible, examination by a wood anatomy specialist and modern species distribution patterns indicate La Brea specimens are either *J. occidentalis* or *J. californica* (Ward *et al.*, 2005). At La Brea, wood specimens are well preserved in their original organic state. The quality of these specimens allows for high-resolution analysis of tree rings and oxygen isotope measurements on individual rings of each tree. In addition, glacial *Juniperus* specimens can be compared to modern individuals from the same native habitat. Modern *Juniperus* samples were obtained by coring *Juniperus* currently growing in Angeles and San Bernardino National Forests. The Nippert *et al.* (2010) model was developed from *J. californica* samples from Lyttle Creek near Fontana, California, which is a low elevation site (629 m) site with hot and dry growing conditions (mean annual precipitation 389 mm, mean annual temperature 18.2 °C; Daly *et al.*, 2008). In order to incorporate a wider range of environmental

conditions, this study included mid- and high-elevation trees with wetter and/or cooler growing conditions. The modern mid-elevation site (1330 m elevation; 2 *J. californica* samples) is also relatively dry (mean annual precipitation 242 mm), but cooler than Lyttle Creek (mean annual temperature 14.7 °C; Daly *et al.*, 2008). The modern high-elevation sites (2340 m elevation; 3 *J. occidentalis* samples) reflect wetter (mean annual precipitation 696 mm) and cooler (mean annual temperature 7.3 °C; Daly *et al.*, 2008) growing conditions, which are comparable to glacial La Brea growing season conditions (Heusser, 1998). The inclusion of these additional modern trees not only allows us to test the model against the broad range of environmental conditions under which *Juniperus* currently grow, but also allows us to directly test growing conditions similar to that of the last glacial period.

Stable Isotope Analysis

Oxygen isotope analysis was performed on alpha-cellulose (which is void of exchangeable oxygen) extracted from individual rings of modern and glacial *Juniperus*. Alpha-cellulose was extracted from whole ring wood using the process described by Ward *et al.* (2005), which has documented high purity levels and no indication of asphalt contamination from the preserving tar matrix. Whole annual rings were separated under a dissecting microscope and analyzed individually to provide an integrated value over the entire growing season. Oxygen isotope analysis was performed at the Keck Paleoenvironmental and Environmental Stable Isotope Laboratory (KPESIL) at the University of Kansas. Results are reported in delta notation using the accepted Vienna standard mean ocean water (VSMOW).

Ring Width Analysis

Ring width was also measured on each of the glacial and modern *Juniperus* specimens. Prior to ring separation, each *Juniperus* sample was imaged at high resolution. From these images, ring widths were measured to the nearest 0.1 mm. Compression of rings during growth can result in variable ring width depending on where in the cross section of the tree the ring is measured. For glacial samples, we are unable to account for such variation, as we have only what limited sections of wood were preserved in the tar pits. For modern samples, we accounted for this by coring each tree on the south side and choosing only the straightest trees. Straight trees exhibit reduced ring compression and trees from the same location show similar compression patterns based on slope and aspect for which coring on the same side of the tree would account.

Correlating Oxygen Isotope Signatures with Climate

To determine how oxygen isotope signatures within modern trees correlated with climatic factors, rings of modern trees were first associated with specific calendar years. Ring width patterns from trees within the same site were correlated and aligned using marker years of high precipitation and growth. Vapor pressure deficit (VPD) was then calculated for each modern growing location. VPD integrates temperature and precipitation effects on evapotranspiration and can correlate more strongly with $\delta^{18}O$ signatures of tree rings than temperature or precipitation alone (ex Ferrio & Voltas, 2005; Roden *et al*, 2005). To calculate VPD, monthly temperature and precipitation data were obtained from the PRISM model, which accurately models climatic conditions in mountainous coastal regions with complex topography (Daly *et al.*, 2008). VPD was calculated from monthly average maximum (T_{max}), minimum (T_{min}), and dewpoint (T_{dew}) temperatures using the equation:

$$VPD = \frac{e_s(T_{\max}) + e_s(T_{\min})}{2} - e_s(T_{dew})$$

where $e_s(T)$ is the saturation vapor pressure at temperature T, calculated as:

$$e_s(T) = 0.6112 \exp\left(\frac{17.67T}{T + 243.5}\right)$$

Statistical Analyses

Since numerous rings sampled from within the same individual tree can exhibit autocorrelation, nested ANOVAs were used to compare $\delta^{18}O$ patterns in glacial and modern *Juniperus*. Tree ID was treated as a random variable, nested within the fixed variable of time period (glacial or modern). For correlations with VPD, linear regression was performed on $\delta^{18}O$ and monthly or annual VPD. For seasonal VPD comparisons, months were grouped as November-December-January (winter), February-March-April (spring), May-June-July (summer), and August-September-October (fall) and compared to $\delta^{18}O$ using a standard least squares linear model. All statistical analyses were performed using JMP 5.1.

Results and Discussion

In this study, we applied a previously-developed El Niño prediction model (Nippert *et al.*, 2010) to modern *Juniperus* trees growing in the San Bernardino mountains of southern California. By testing the model against higher-elevation trees that experience cooler and wetter growing conditions, we intended to validate the model's predictive power under a variety of growing conditions. If the model accurately predicts El Niño events in these additional modern *Juniperus* samples, it can then be applied to glacial *Juniperus* remains obtained from the La Brea tar pits, allowing us to reconstruct ENSO patterns over the last 50,000 years in this region.

The Nippert *et al.* (2010) model relied upon a positive relationship between ring width and $\delta^{18}O$ in El Niño years, and a negative relationship between ring width and $\delta^{18}O$ in non-El Niño years due to different drivers of oxygen fractionation under El Niño and non-El Niño conditions (Fig. 3.1, see Materials and Methods for more details). This underlying relationship is only weakly present in mid- and high-elevation *Juniperus* (Fig. 3.3), which renders the Nippert *et al.* (2010) model unable to accurately predict El Niño years in these trees.

In order to strengthen the predictive power of the model on the mid- and high-elevation modern *Juniperus*, a number of additional parameters were considered. First, years were split into three classifications (El Niño, neutral, and La Niña) as opposed to only two (El Niño and non-El Niño) as combining neutral and La Niña years may have contributed to the weakened relationship in the non-El Niño category. Second, instead of grouping all modern trees for ring width/ $\delta^{18}O$ analysis, each site was analyzed separately, and each individual tree separately. Lastly, we scaled the $\delta^{18}O$ signatures of each modern *Juniperus* tree by elevation, adjusting each tree ring $\delta^{18}O$ value to a 'low-land equivalent' based on the elevation at which the individual tree grew. Studies in southern California have identified an elevational gradient of the isotopic signature of precipitation, on the order of -0.17‰ per 100 m increase in elevation (Poage & Chamberlain, 2001). This gradient means that mid- and high- elevation trees experience precipitation that is 2.3-3.9‰ lighter (depleted in ^{18}O) than that experienced at the low elevation site used to develop the original model. Since source water $\delta^{18}O$ signatures can impact plant tissue $\delta^{18}O$ signatures (e.g. Danis *et al*, 2006), this variation could partially mask the El Niño/non-El Niño trend necessary for consistent El Niño prediction by the model. Unfortunately, none of these additional considerations served to increase the predictive power of the model for mid- and high-elevation modern *Juniperus*. Consequently, it appears that mid- and high-

elevation *Juniperus* currently growing in the Los Angeles Basin exhibit fundamentally different ENSO-physiology relationships than *Juniperus* growing in lower elevation areas in the same region.

In order to understand why mid- and high-elevation modern *Juniperus* exhibit different ENSO-physiology relationships than the low-elevation trees used for the Nippert *et al.* (2010) model, $\delta^{18}O$ and ring width patterns in these trees were examined in more detail. Generally, increasing water stress produces higher $\delta^{18}O$ signatures in tree ring cellulose (Roden *et al.*, 2000; Ferrio & Voltas, 2005), though this trend is not always present (e.g. Roden *et al.*, 2005; Roden & Ehleringer, 2007). Evaporation at the leaf increases $\delta^{18}O$ because $H_2{}^{16}O$ molecules evaporate more readily than $H_2{}^{18}O$ molecules, producing an effect termed evaporative enrichment. Under high transpiration levels this effect is actually lessened because evaporated water is replaced by non-enriched water from the plant stem (termed the Péclet effect; Sternberg, 2009). Consequently, higher enrichment effects occur under high VPD, but low transpiration levels representing more conservative stomatal control under drought conditions (Sternberg, 2009).

Modern trees from mid and high elevations did not show statistically different $\delta^{18}O$ values, despite differences in precipitation and temperature between these sites (Fig. 3.4; mid elevation mean 31.6 ± 0.2; high elevation mean 31.6 ± 0.2, $p = 0.92$). Modern mid- and high-elevation *Juniperus* did not show significantly different $\delta^{18}O$ signatures compared to the low-elevation Lyttle Creek trees used to develop the Nippert *et al.* (2010) model despite higher water stress at low elevations (low elevation mean: 31.7 ± 0.2; $p = 0.88$). Additionally, all but one modern mid- and high-elevation *Juniperus* exhibited no significant trends between $\delta^{18}O$ and VPD on monthly, seasonal, or annual scales, indicating negligible impacts of water stress on $\delta^{18}O$. Despite constant $\delta^{18}O$ across modern *Juniperus,* glacial $\delta^{18}O$ signatures were significantly

lower than those of modern *Juniperus* (glacial mean 30.1 ± 0.3; modern mean 31.6 ± 0.3; p = 0.005). This trend might be expected for the mid-elevation *Juniperus*, which experience increased water stress compared to glacial *Juniperus*; however, high-elevation *Juniperus* were selected for their comparableness to glacial conditions, making a significant difference surprising. A previous study reported similarly confounding data, whereby *Juniperus* did not reflect the expected increase in $\delta^{18}O$ under increased water stress compared to other conifer species growing in less water-stressed environments (Roden *et al.*, 2005). In that study, the lack of increase was attributed to *Juniperus* ring wood being laid down earlier in the growing season, when water stress is less pronounced (Roden *et al.*, 2005). These trends may also be due to the anisohydric hydraulic nature of *Juniperus*, whereby trees maintain water uptake even under water-stressed conditions (West *et al.*, 2007). An anisohydric hydraulic strategy could thereby increase the influence of the Péclet effect, reducing the evaporative enrichment of $\delta^{18}O$ in plant tissue. Additionally, when climatic conditions do not strongly limit tree growth, individual tree effects can show stronger impacts on $\delta^{18}O$ than environmental parameters (Reynolds-Henne *et al.*, 2009). Consequently, as we move from low- to high-elevation *Juniperus* growing environments (corresponding with a shift from high to low water stress), we may see an increasing influence of individual tree effects on $\delta^{18}O$. Additional work would be needed in order to directly test the influence of these factors on this system.

It had been suggested, even at the time of its development, to apply the Nippert *et al.*, model to glacial *Juniperus* trees in order to reconstruct paleo-ENSO dynamics. Unfortunately, the lack of predictive power of the model for modern *Juniperus* outside the original site location suggests the model may not accurately predict El Niño conditions in glacial *Juniperus*. Still, the physiological trends presented here can shed some light on glacial *Juniperus* climate-physiology

relationships. We cannot directly test for the underlying ENSO relationship between ring width and $\delta^{18}O$ in glacial trees, namely the positive relationship in El Niño years and negative in non-El Niño years (Fig. 3.1); however, the weakening of this relationship in mid- and high-elevation modern *Juniperus* suggest that under less limiting growing conditions (i.e. cooler and wetter), *Juniperus* do not respond as strongly to El Niño-induced alterations in climate. The significantly lower glacial $\delta^{18}O$ values might at first appear to suggest a reduction in water stress in glacial *Juniperus*. The last glacial period was cooler and wetter in Rancho La Brea than it is today (Heusser, 1998), however, modern trees were selected specifically for comparable environmental conditions as the last glacial period. The fact that modern trees do not exhibit a $\delta^{18}O$ increase with increasing water stress suggests the climate-physiology relationship is more complicated in *Juniperus* than a simple water stress model might imply. The responses described here, and presented in past research (Roden *et al.*, 2005) illustrate the counter-intuitive nature of *Juniperus* responses to environmental factors. These responses must be assessed in more detail before glacial *Juniperus* could be utilized in paleoclimatic reconstructions of this nature.

Chapter 3 Figures

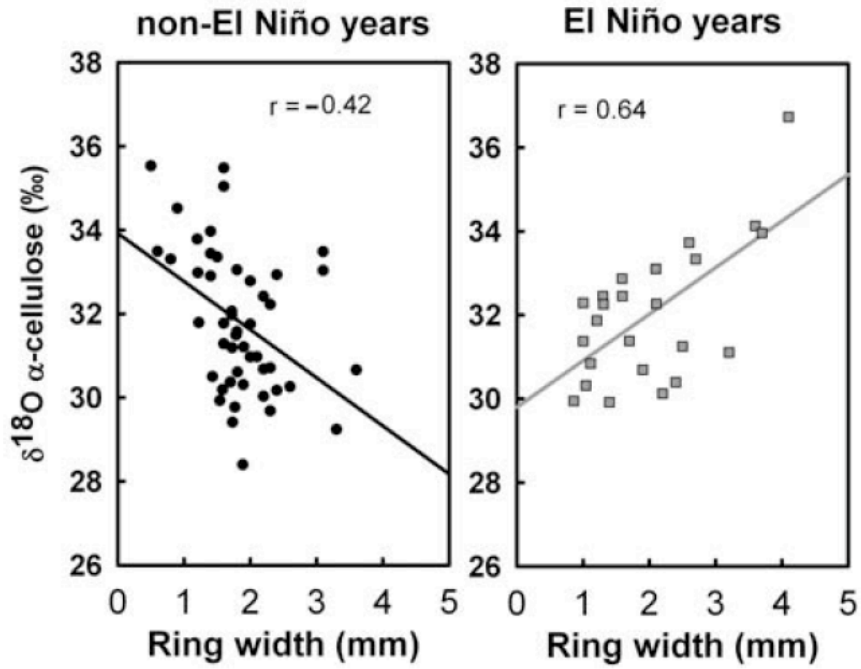

Figure 3.1. Changes in the stable isotopic signature of oxygen ($\delta^{18}O$) in alpha cellulose correlate with tree ring width during non-El Niño and El Niño years. Data reflect local environmental conditions recorded in juniper tree rings from 1969-2003. Taken from Nippert *et al.*, 2010.

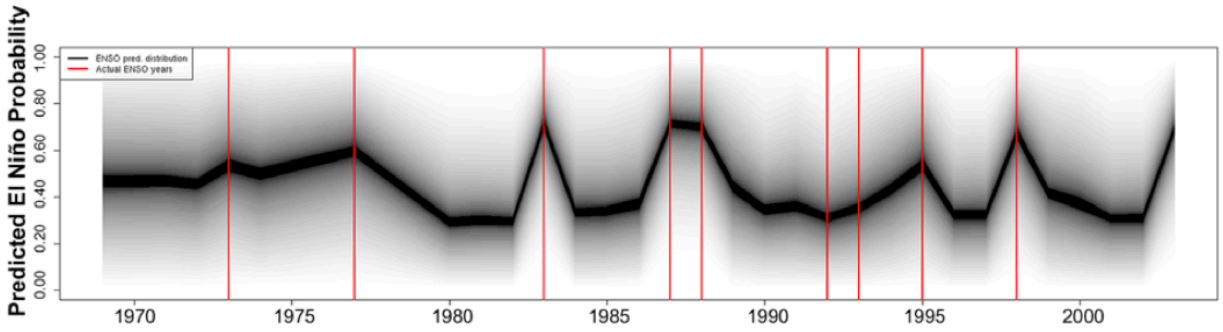

Figure 3.2. Cross-validation of predictions of El Niño probability. Each year of data was sequentially withheld for model fitting, and then the probability of an El Niño year was predicted. Solid vertical red lines illustrate actual El Niño years during this period. The black line represents the mode of the predictive distribution of El Niño probability over the years. The gray shading represents the predictive distribution itself, where darker gray indicates areas of higher probability density (i.e., likely) and lighter values indicate lower-density areas (i.e. unlikely). Years where the gray shading is more spread out indicate a higher prediction variance (i.e., less certainty in the prediction). Taken from Nippert *et al.*, 2010.

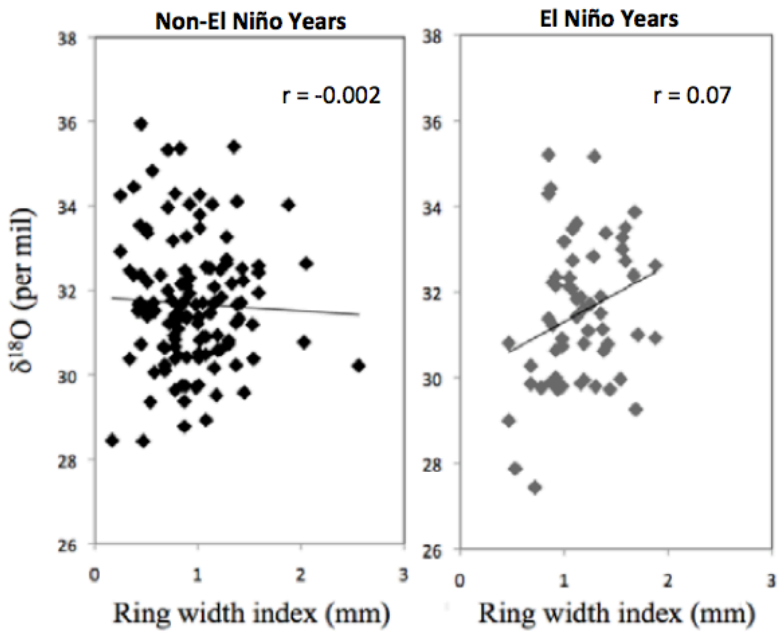

Figure 3.3. Oxygen isotope composition and ring width index for modern mid- and high-elevation *Juniperus* used to test the Nippert *et al.* (2010) model at conditions of cooler temperature and higher precipitation, characteristic of the last glacial period. Non El Niño years include both neutral and La Niña years. Data are presented here in the same format as Figure 3.1 (the original Nippert *et al.* 2010 data) for ease of comparison.

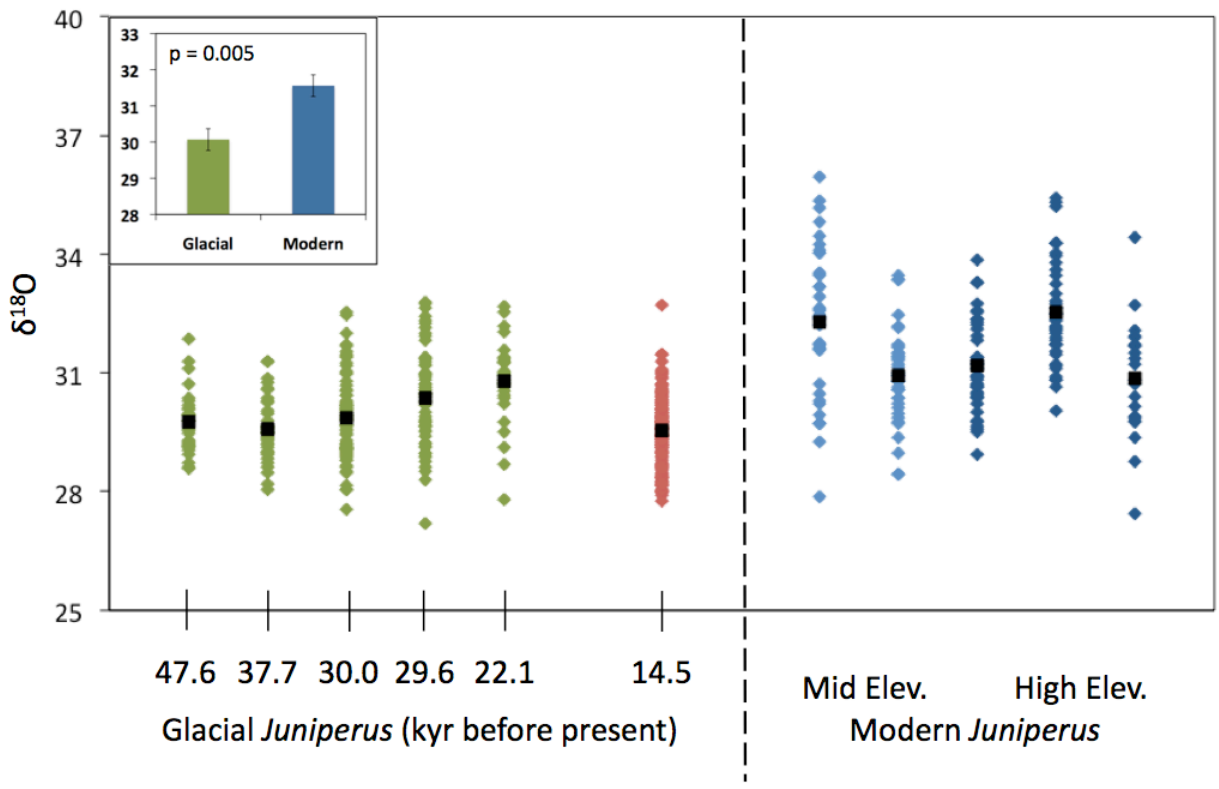

Figure 3.4. Oxygen isotope signatures of glacial and modern *Juniperus* tree rings. Each point represents a single annual tree ring, and vertical groups represent all available tree rings for an individual tree, with values stacked highest to lowest. Glacial samples are shown in green and the one Holocene age individual is shown in red. Modern mid-elevation trees are shown in light blue and modern high elevation trees shown in dark blue. In all cases, the mean for each tree is represented by a black box. The inset shows group means for glacial and modern *Juniperus*, with error bars of one standard error.

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The following chapter stems from the University of Kansas National Science Foundation IGERT (Integrative Graduate Education and Research Traineeship) program titled C-CHANGE: Climate Change, Humans, and Nature in the Global Environment. The C-CHANGE program trains students on interdisciplinarity in climate change science, including a science policy internship intended to focus students on the policy implications of their research. This chapter was inspired by the author's internship with the Office of Government Relations at the American Association for the Advancement of Science in Washington D.C. in the fall of 2012.

Chapter 4: *Massachusetts vs EPA*: History and Future of a Landmark Case

Introduction

In April of 2007, the United States Supreme Court formally determined that carbon dioxide (CO_2) along with six other greenhouse gases (GHGs) together constituted a single air pollutant to be regulated by the Environmental Protection Agency (EPA) under the authority of the Clean Air Act (CAA). For many Americans, this was likely the first they had heard of the potential to regulate CO_2 in such a way; however, EPA had been considering this strategy for nearly a decade (Heinzerling, 2007). The Supreme Court decision, and the resulting vehicular fuel efficiency standards, marked a significant shift in the federal approach to climate change* legislation, and initiated or enhanced similar shifts in corporate institutions throughout the U.S.

**Note: Both 'climate change' and 'global warming' are used throughout this article. While there are important scientific differences in these terms, they are often used interchangeably in the public and political realms. When referencing a specific document or survey, this article uses the same terminology as the reference; otherwise, the term 'climate change' is used in order to incorporate all aspects of change that may occur.*

This case, therefore, offers a unique opportunity to delve into the push and pull in science policy, and how the public and policymaker's understanding of complex scientific ideas translate into effective regulatory policies.

This paper will address the following overlapping and interconnected issues surrounding the *Mass v EPA* court case: 1) the complex history of EPA's attitude towards classifying CO_2 as an air pollutant, 2) the impact of the *Mass v EPA* court case in the context of U.S. climate change legislation in the federal government and state governments, as well as in institutional policy in the military and corporate worlds, 3) the *Mass v EPA* decision as a case study in how scientific knowledge is filtered through a political lens, 4) the impact of public understanding of climate change issues on science policy, and 5) the importance, difficulty, and hesitation surrounding scientists' communication with the public and policymakers. Generally, the paper will focus on nation-wide patterns, but where applicable, the role of Kansas legislators and the impact of climate change legislation on Kansas industry will also be discussed.

History of EPA Opinion on CO_2

The 2007 *Mass v EPA* court case was a landmark event in climate change litigation and legislation and received ample media attention and public discussion. This case, however, was far from the first political discussion of whether or not CO_2 should be classified as an air pollutant. By the time the public was aware of this debate, EPA had been analyzing CAA wording, Congressional intent, and regulatory ramifications for nearly a decade. This section will provide a detailed overview of the EPA debate over CO_2, including key events, critical players, and shifts in strategy, and will focus on the complexity surrounding this issue to provide the reader a broader picture of the CO_2 air pollutant debate.

In 1998, then General Council of EPA, Jonathan Cannon issued a memorandum in response to a request from Congressman Tom DeLay (R, Texas) for a legal opinion from EPA on classifying CO_2, nitrogen oxides (NO_x), sulfur dioxide (SO_2) and mercury as pollutants (Cannon, 1998). The official finding of the Cannon Memorandum was that CO_2 is an air pollutant covered under the CAA and subject to regulatory management by EPA. In fact, of the list of compounds in Congressman DeLay's request, EPA already regulated each substance except CO_2 under the authority of the CAA (Cannon, 1998). The Cannon Memorandum cited numerous sections of the CAA under which CO_2 could reasonably be regulated, if the Administrator (then Carol Browner, appointed by President Bill Clinton in 1993) formally determined that CO_2 endangered public health or welfare. Cannon emphasized the "preventative and precautionary" nature of the CAA and its intention to "prevent harm before it occurs" as well as Congress' broad and inclusive language throughout the Act. CAA Section 302(g) defines an air pollutant as "any air pollution agent or combination of such agents, including any physical, chemical, biological, [or] radioactive… substance or matter which is emitted into or otherwise enters the ambient air." In Section 108(a)(1), the CAA grants EPA authority over "air pollution which may reasonably be anticipated to endanger public health or welfare" and CAA Section 302(h) specifically lists effects on weather and climate as an impact on welfare (CAA, 2004). In addition, Section 103(g) specifically lists CO_2 in a list of air pollutants, though this list is in reference to non-regulatory strategies of research and technology development (CAA, 2004). After reviewing these sections, Mr. Cannon determined that CO_2 is an air pollutant within the context of the statute and subject to EPA regulation (Cannon, 1998).

Though the Cannon Memorandum focused mainly on the legal framework of the CAA, some biological considerations were also included. Though CO_2 is naturally present in the

atmosphere and is emitted from natural, as well as anthropogenic sources, Cannon argued it could still be considered an air pollutant under the CAA since numerous other compounds, including SO_2, volatile organic compounds, and NO_x are naturally occurring, EPA regulated air pollutants (Cannon, 1998). Cannon also noted that EPA regulates other substances that are "actually necessary in trace quantities for human life, but are toxic at higher levels" and for which "human activities have increased the quantities present in the air to levels that are harmful to public health, welfare, or the environment" (Cannon, 1998). The opinion of the Cannon Memorandum was supported by Gary Guzy, Cannon's successor as EPA General Council, who testified before Congress on the issue in 1999 (Fabricant, 2003). Though Guzy noted that EPA Administrator Browner had yet to make any formal determinations to regulate CO_2 under the specific provisions of the CAA, he stated "there is no statutory ambiguity" concerning the authority of EPA to regulate CO_2 under this statute (Fabricant, 2003).

In 1999, nearly 20 organizations led by the International Center for Technology Assessment (ICTA) petitioned the EPA to regulate GHG emissions from motor vehicles under section 202(a)(1) of the CAA (Mendelson, 1999). The petition cited the contribution of GHG emissions to global warming, which the petitioners argued was already affecting public health and welfare. Many of the arguments the petitioners made were similar to those put forth in the Cannon Memorandum a year earlier (Cannon, 1998; Mendelson, 1999) Since vehicles accounted for over 20% of CO_2 emissions in the United States, petitioners argued reductions in emissions in this sector could strongly impact global warming. In response to this petition, new EPA Administrator Christine Todd Whitman, only recently appointed by President George W. Bush, published a Notice and Request for Comments in the Federal Register on January 23, 2001 titled "Control of Emissions from New and In-use Highway Vehicles and Engines," requesting public

input on the issues raised in the petition, and which was open to public comment for four months (Perciasepe, 2001).

The Notice generated over 50,000 public comments, which were under review by EPA for over 18 months before ICTA and other environmental groups filed a lawsuit against EPA to compel a response to the petition (*ICTA v Whitman*, 2002). During the two and a half years since filing the original Notice, Administrator Whitman had become increasingly frustrated with the right-leaning stances of the Bush Administration, and resigned her position as EPA Administrator in May 2003 (she would later publish a memoir about her disenfranchisement with the Republican Party). President Bush then appointed acting Administrator Marianne Lamont Horinko, who concluded that GHG emissions standards for motor vehicles were not appropriate at this time. The lawsuit from ICTA was settled when EPA submitted a Notice of Denial of Petition for Rulemaking on September 8, 2003 (Holmstead, 2003). The Notice of Denial cited global change implications on foreign policy, for which the President is responsible, as well as Congressional silence in CAA amendments and other legislation on motor vehicle standards, which the Notice interpreted as a lack of Congressional intention for EPA regulation of GHGs (Holmstead, 2003). In addition, the Notice highlighted the "substantial scientific uncertainty" and the "paucity of data" surrounding climate change issues and impacts and the need for increased research before any climate change related policies are enacted by any agency or governmental branch (Holmstead, 2003). The Notice then described numerous voluntary and nonregulatory approaches to reducing GHG emissions put forth by the EPA and the Bush Administration and the positive impacts these measures have had on reducing GHG emissions (Holmstead, 2003). The decision against regulation was further justified, the Notice claimed, by the argument that regulation by EPA would conflict with standards implemented by the

Department of Transportation under the Energy Policy Conservation Act. Since EPA regulation under Section 201 is discretionary, even if it were determined the EPA had authority to regulate, Administrator Horinko indicated it would decline to exercise that authority (Holmstead, 2003).

As a more public response to the ICTA petition, EPA General Council Robert Fabricant issued a memorandum representing the new EPA stance on CO_2 regulation (Fabricant, 2003). The Fabricant Memorandum overturned the previous opinions of Cannon and Guzy concluding that the CAA did not grant EPA regulatory authority over global climate change issues and so, EPA could not exert jurisdiction over GHG emissions. In addition, Fabricant argued, the CAA authorizes EPA to develop non-regulatory measures of research and technological development, but does not indicate that EPA should regulate global climate change issues. Fabricant also argued the authority the EPA does have for setting national ambient air quality standards (NAAQS) would be ineffective against CO_2. NAAQS have been effective in the past for compounds that are near the surface of the earth and unevenly distributed across space; however, since CO_2 is well-mixed throughout the atmosphere, no region within the United States could be in compliance with CO_2 NAAQS until the entire world was in compliance. Consequently, Fabricant argued, the NAAQS system is fundamentally not suited to regulating CO_2. In addition, Fabricant felt that Congress did not intend EPA to regulate global climate change under broad CAA language, but instead to wait for a specific provision from Congress. For these reasons, Fabricant concluded that CO_2 and other GHGs could not be determined air pollutants under the CAA's regulatory provisions.

Approximately a month later, in October of 2003, the state of Massachusetts led a group of twelve additional states, two cities, American Samoa, the District of Columbia, and fourteen non-governmental public interest organizations in challenging the Rulemaking Denial Notice as

well as the Fabricant Memorandum in the District Court of the District of Columbia (hereafter, D.C. District Court; Winters, 2004). Respondents in this case included EPA (against whom the case was originally filed), as well as ten states and 19 industry and utility groups, who intervened in the case on behalf of EPA (Osofsky, 2008). Then Kansas Attorney General Phil Kline, along with eight other state Attorney Generals filed a brief supporting EPA's stance against CAA-based GHG regulation (Cox *et al.,* 2006). This brief supported the primary argument of the Fabricant Memorandum, namely that a globally mixed compound like CO_2 could not be adequately regulated using NAAQS, and that reducing GHG emissions in the United States would serve only as 'an exercise in futility' to reducing U.S. air pollution and protecting public health and welfare (Cox *et al.,* 2006). The brief accused the petitioners of ignoring core provisions of the CAA, and using 'tunnel vision' in their interpretations of Section 202(a)(1) and argued that the CAA, when viewed in its entirety, clearly does not authorize EPA to address global climate change (Cox *et al.*, 2006). In July 2005, after nearly two years of litigation and two changes in EPA Administrators (though still under the Bush Administration), the D.C. District Court ruled against the petitioners (*Mass v EPA*, 2005), who then appealed to the Supreme Court. On April 2, 2007, the Supreme Court voted 5-4 in favor of the petitioners and held that the CAA gives EPA the authority to regulate GHGs and that EPA could not decline to exercise this authority based on policy considerations or general scientific uncertainty (*Mass v EPA*, 2007). The Supreme Court determined the plain language of the CAA was unambiguous on the issue and that agencies have as much of an obligation to avoid failing to regulate as they do to unlawfully regulate (*Mass v EPA*, 2007). The Supreme Court ruling ordered EPA to review the scientific evidence and determine if GHGs posed a danger to human health or the environment.

Over a year following the Supreme Court ruling, EPA had made no such determination. Consequently, Massachusetts and the other petitioners again filed suit against EPA requesting the DC Circuit Court to issue a writ of mandamus requiring EPA to make a formal determination on whether or not GHG emissions from motor vehicles endanger public health and welfare (*Mass v EPA*, 2008). This petition was denied by the court without explanation, effectively giving EPA an unlimited amount of time to review the potential dangers of GHG emissions. Under Congressional pressure, EPA (now under Administrator Stephen Johnson, appointed by G.W. Bush) issued an Advance Notice of Proposed Rulemaking (ANPR) on July 30th, 2008 (Johnson, 2008). The ANPR made no formal determination on GHG dangers (as required by the Supreme Court), but instead solicited public comments on GHG regulation under the CAA and proposed regulatory mechanisms (Johnson, 2008). Interestingly, while the text of the ANPR itself discussed the statutory language of the CAA to act preemptively to prevent harm to human health and the environment, the opening statement from Administrator Johnson argued the CAA was not the ideal legal framework (while simultaneously requesting public input on that very issue) and stated that CAA-based GHG regulation "would be relatively ineffective at reducing greenhouse gas concentrations given the potentially damaging effect on jobs and the U.S. economy" (Johnson, 2008). This is especially interesting wording, considering EPA had been expressly forbidden by the Supreme Court from making regulatory decisions based on political considerations and not scientific research.

The ANPR comment period closed in late November, 2008 during the lame duck period after Barack Obama had been elected president, but while President Bush was still in office. Soon after taking office in January of 2009, President Obama made clear his intention to address the CO_2 regulatory issue and by April 24, 2009 Obama-appointed EPA Administrator Lisa

Jackson issued two findings under Sec 202(a) of CAA. The first, commonly referred to as the Endangerment Finding determined a mix of six GHGs, including CO_2, to be endangering to public health and welfare of current and future generations (Jackson, 2009a). The second, the Cause or Contribute Finding, addressed how these compounds cause or contribute to the threat of climate change. Citing numerous scientific studies on climate change and the potential impacts these changes may have on human health, Administrator Jackson determined that "the evidence points ineluctably to the conclusion that climate change is upon us as a result of greenhouse gas emission, that climatic changes are already occurring that harm our health and welfare, and that the effects will only worsen over time in the absence of regulatory action" (Jackson, 2009a). Consequently, the findings determined that six greenhouse gases – carbon dioxide, methane, nitrous oxide, hydrofluorocarbons, perfluorocarbons and sulfur hexafluoride – together constituted a single air pollutant to be regulated by EPA under Section 202(a)(1) of the CAA.

Regulatory Impacts

The conclusion of *Mass v EPA* opened the door for EPA regulation of light duty vehicle tailpipe emissions. Despite nearly a decade of arguments over the science behind GHG emissions, the political impacts of regulation, and the specific wording and intent of the CAA, the Supreme Court finalized the discussion forcing EPA to review the scientific literature of GHG emissions and ignore political considerations. The Endangerment and Cause and Contribute Findings were far from the end of the issue, however. These Findings set the stage for numerous rules and regulations from EPA, which had a cascading effect for court cases questioning the regulations, Congressional hearings over economic impacts, and even set precedent for state-level GHG emission decisions. This section will discuss the legal and

political ramifications of the Supreme Court decision in the five years following the conclusion of *Mass v EPA*.

As a direct result of the *Mass v EPA* decision and the subsequent Endangerment Finding, EPA and the Department of Transportation issued a joint rule to increase fuel efficiency standards in light duty vehicles under the Corporate Average Fuel Economy (CAFÉ) standards under the Energy Policy and Conservation Act (LaHood & Jackson, 2010). These standards, known as the Tailpipe Rule, went into effect in January 2011 and increased average fuel efficiency to 35.5 miles per gallon for model years 2012-2016, reducing U.S. CO_2 emissions by an estimated 960 million metric tons and saving an estimated 1.8 billion barrels of oil over the lifetime of the vehicles (LaHood & Jackson, 2010). In 2011, similar fuel efficiency standards were enacted for medium and heavy duty vehicles, saving an estimated 270 million metric tons of CO_2 emissions and 530 million barrels of oil (LaHood & Jackson, 2011). Additionally, the light duty vehicle standards were later extended to include model years 2017-2025, saving an additional 2 billion metric tons of CO_2 emissions and 4 billion barrels of oil (LaHood & Jackson, 2012).

Though *Mass vs EPA* specifically focused on regulation of mobile, vehicular sources of GHG emissions, stationary sources are covered by the same CAA language, which opened these sources to regulation as well. In 2009, EPA instituted mandatory reporting of GHG emissions for the nation's largest stationary sources (Jackson, 2009b), and later amended these rules to include all petroleum and natural gas systems (Jackson, 2010c). In 2010, EPA also determined that major stationary GHG sources must obtain construction and operating permits limiting GHG emissions. In order to limit the regulatory burden on these sources, EPA issued the Timing Rule, which determined when GHG regulation by EPA is initiated for a particular source, and the

Tailoring Rule, which determined which sources are subject to permitting (Jackson, 2010a,b). A Prevention of Significant Deterioration (PSD) permit is required prior to construction of any new facility, or prior to alterations to existing facilities that would significantly increase emissions (Jackson, 2010a). Under the Timing Rule, PSD permitting is not triggered until a given pollutant is covered under a final nation-wide ruling, and not before that rule takes effect (allowing unregulated emissions while the rule is under consideration, including publication in the *Federal Register*; Jackson, 2010b). Under the Tailoring Rule, PSD permitting is not triggered until threshold emissions levels for a given pollutant are exceeded. This rule, therefore 'tailors' the PSD permitting requirements to affect only the nation's largest GHG emitters – namely power plants, refineries, and cement production – with the intention of later lowering this threshold to include smaller facilities (Jackson, 2010b).

EPA rulings and the *Mass v EPA* decision were also used as precedent for state-level regulations of stationary source GHG emissions. For example, then Kansas Governor Kathleen Sebelius and Kansas Department of Health and Environment (KDHE) Secretary Rodrick Bremby used the *Mass v EPA* ruling in an attempt to halt the construction of coal plants by Sunflower Electric in Holcomb, KS (Blanton et al, 2008). In 2007, Sunflower had requested permits for the addition of two new 700 megawatt (MW) coal-fired steam generating units to an existing plant in Holcomb; however, the permits were denied by Secretary Bremby due to the negative impacts of climate change resulting from CO_2 emissions (Bremby, 2007). This decision marked the first time climate change was directly cited as the reason for denying such a permit. Prior to the decision, Secretary Bremby requested legal counsel from then Kansas Attorney General Paul Morrison. After reviewing Kansas statutes, AG Morrison determined that the Secretary was legally allowed to deny an air quality permit if he first made a factual

determination that a particular emission presented a danger to the environment or public health (Morrison & Nohe, 2007). AG Morrison also noted, however, that 'it is highly questionable whether such an action would survive a due process challenge, or a challenge based upon such action being arbitrary, capricious, and unreasonable" (Morrison & Nohe, 2007). Bremby's decision was also supported by an open letter from eight other State Attorney Generals strongly encouraging Kansas to 'explore alternatives' in its energy portfolio and not undo the advances made in other states for reducing GHG emissions (Spitzer *et al*, 2006). The denial of Sunflower permits sparked a heated response, including three bills from the 2008 Kansas legislature to overturn the decision, each of which was vetoed by Governor Sebelius (Glicksman, 2008). The Kansas House of Representatives considered filing a lawsuit against Governor Sebelius and Secretary Bremby for violating separation of powers between the executive and legislative branches (H.C.R. 5042), though this resolution was never brought to a vote. Sunflower Electric, however, did file suit against Governor Sebelius, Lieutenant Governor Mark Parkinson, and Secretary Bremby in 2008 for violation of Sunflower's Constitutional rights, including equal protection under the 14th Amendment, and the Commerce Clause (*Sunflower Electric v Sebelius et al*, 2008). While this case was under review by the Kansas District Court, President Obama appointed Governor Sebelius to the position of Secretary of the Department of Health and Human Services in Washington, D.C. In her absence, Lieutenant Governor Mark Parkinson became the interim governor and reached a consent agreement with Sunflower Electric for the construction of one 895 MW ultra-super critical coal (i.e. 'clean coal') power plant (Parkinson & Watkins, 2009). As part of this agreement, Sunflower was also required to develop a 179 MW wind farm, burn 10% biomass at both the new and existing plants in Holcomb, and provide $4 million in annual funding to Kansas energy efficiency programs (Parkinson & Watkins, 2009).

This agreement was reached less than one week after Governor Parkinson was sworn into office, and later, approval of the permits was hastened for completion before the end of the 2010 calendar year, when the previously-mentioned EPA Timing Rule would go into effect, triggering additional GHG emission controls for the plant (Dillon, 2010). These hastened timelines, coupled with the resignation of Secretary Bremby in November 2010 led some to question the relationship between the Kansas legislature and Sunflower Electric (Dillon, 2010; Holman, 2010). Sunflower's air quality permit for the Holcomb expansion was approved on Dec 16th, 2010, though two years later, it does not appear Sunflower has begun construction of the new facility or any of the additional requirements of the agreement (Holcomb, 2012).

Other states also used the ruling to address GHG emissions. Florida denied a construction permit for a $5.7 billion coal-fired power plant requested by Florida Power and Light (FPL) based on concerns over future costs associated with regulation of GHG emissions from such power sources (FPSC, 2007). FPL requested two new 980 MW power units in the Glades Power Park, west of Lake Okeechobee, near the Florida Everglades (FPSC, 2007). Though the decision was not explicitly based on the dangers of global warming (as it was in Kansas), the increased permitting costs of coal-fired plants due to realized and potential future legislation still effectively shut down the construction permit. In a rather unusual turn of events, private equity firms bought out TXU Corp (Texas' largest energy provider) in 2007 after the company announced plans to build 11 new coal-fired power plants in Texas by 2010 (Mufson & Cho, 2007). The $45 billion buyout was developed in collaboration with environmental groups, such as the Environmental Defense Fund, and included the elimination of eight of the 11 planned power plants (Mufson & Cho, 2007) as well as a corporate pledge to support mandatory carbon emissions caps (EDF, 2007). In addition, numerous states have pursued renewable portfolio

standards (RPSs) to increase use of renewable fuels and reduce GHG emissions. As of February 2013, 30 states and the District of Columbia have adopted mandatory RPSs and an additional seven states have set voluntary goals (EIA, 2012). Though there is currently no national RPS, the proliferation of state-level action effectively pushes the United States towards a *de facto* national standard, albeit through a patchwork of regional standards of varying stringency.

After their implementation, EPA regulations were quickly tied up in federal courts where the Endangerment Finding and related rules were challenged numerous times (for a survey of climate change litigation in the U.S., see Markell & Ruhl, 2010). In 2011, *Texas v EPA*, challenged the impacts of the PSD permitting program on state permitting programs and state implementation plans of new source GHG permits. This case was dismissed by the DC Circuit Court due to lack of standing to sue and a failure to prove economic harm from EPA regulations (*Texas v EPA,* 2012). The Center for Biological Diversity also brought suit against EPA seeking to expand the agency's regulation of GHG emissions to include aircrafts, ships, and other non-road engines. In this case, the D.C. District Court determined EPA was required to issue endangerment findings for aircraft GHG emissions, but not for marine, or other non-road engine sources (*CBD v EPA,* 2011). Lastly, the Coalition for Responsible Regulation and other petitioners brought suit against EPA in 2012, challenging the Endangerment Finding, and the subsequent Tailpipe, Timing, and Tailoring Rules. The petitioners argued that these Findings and Rules were arbitrary and capricious constructions of the CAA. Kansas Attorney General Derek Schmidt filed a brief of *amicus curiae* supporting the petitioners, citing 'new evidence that was unavailable to the States and the public during the notice and comment period [for the Endangerment Finding], evidence that casts significant doubt on the integrity and reliability of the "science" on which the EPA relied" (Schmidt, 2011). Specifically, AG Schmidt challenged

the assertion by the U.N Intergovernmental Panel on Climate Change that anthropogenic GHG emissions endanger public health, citing as his evidence the illegal disclosure of private emails and other documents from scientists at the University of East Anglia Climate Research Unit (Schmidt, 2011). The D.C. District Court ruled the petitioners did not have standing to challenge the Timing and Tailoring Rules, that the Endangerment Finding and Tailpipe Rule were not arbitrary or capricious, and that EPA's interpretation of CAA regulation of stationary sources was 'unambiguously correct' (*CRR v EPA*, 2012). Collectively, these cases served to reinforce the courts' support of EPA GHG regulation from both mobile and stationary sources, and upheld all Findings and Rules relating to these regulations.

With its authority to regulate GHG emissions repeatedly supported by judicial opinion, EPA continued to propose new rules, including New Source Performance Standards (NSPS) for electricity-generating plants in March 2012 (Henigen, 2012). These standards would set limits of carbon pollution for any new fossil-fuel-powered electric plant built in the United States, including natural gas and coal. The comment period for the proposed rule closed June 12, 2012; however, as of December 2012, a final rule had not yet been issued for electricity generation. EPA has issued NSPS for petroleum refineries, though it is still unclear when these will go into effect (Jackson, 2012).

Increasing federal regulation of the energy sector prompted the House of Representatives Natural Resources Committee (NRC) to create the American Energy Initiative (AEI), charged with removing federal barriers to energy production and reducing energy prices for American consumers. This initiative sponsored 23 bills in the 112th Congress, relating to a variety of energy sources and issues (see AEI, 2012 for a full listing and up-to-date statuses). These include the Stop the War on Coal Act to halt regulation of GHG emissions and preserve one national

standard for automobiles (H.R. 3409), the Congressional Replacement of President Obama's Energy-Restricting and Job-Limiting Offshore Drilling Plan to expand off-shore oil and natural gas exploration (H.R. 6082), the Putting the Gulf of Mexico Back to Work Act to redevelop oil and natural gas leases in the Gulf of Mexico (H.R. 1229), and the Natural Petroleum Reserve Alaska Access Act to initiate competitive leasing of oil and gas reserves in Alaska through 2021 (H.R. 2150). Many of the AEI-sponsored bills also promoted renewable energy sources, such as the Cutting Red Tape to Facilitate Renewable Energy Act to streamline federal review of renewable energy development (H.R. 2170), the Advancing Offshore Wind Production Act to do exactly what the name implies (H.R. 2173), and the National Strategic and Critical Minerals Policy Act to conduct an assessment of rare earth minerals used in renewable energy production (H.R. 2011). While several of these bills have already passed the House, it is unlikely that many more will be addressed during the lame-duck session by either the House or the Senate. Additionally, the more conservative bills are unlikely to survive a Democrat-controlled Senate. Consequently, few (if any) are likely to be signed into law by the 112th Congress.

In the summer and fall of 2012, the House Committee on Energy and Commerce Subcommittee on Energy and Power (E&P) held numerous AEI hearings, including four relating directly to GHG regulation. These hearings focused generally on EPA's current, future, and pending GHG regulations, regulatory burdens on industry, and impacts of regulation on facility construction and expansion, energy costs, jobs, and the economy. E&P was particularly concerned with regulations relating to carbon capture and storage (CCS) for coal-powered electricity plants. EPA's NSPS for electric plants combined coal- and natural gas-powered utilities into a single source category with a shared CO_2 emission standard (Henigen, 2012), the level of which requires coal plants to incorporate CCS technology for reduction of CO_2

emissions. E&P argued that since CCS technology is not currently commercially viable, the EPA rule imposes 'a de facto ban on any new coal plants' (E&P, 2012) and E&P sponsored legislation prohibiting the EPA Administrator from imposing any such standards until CCS was proven to be technologically and economically feasible (H.R. 6172). This bill was held in committee throughout the lame duck session, but may be pursued by the 113th Congress.

In the midst of all this activity, the Obama Administration also took action on GHG emissions. The Federal Leadership in Environmental, Energy, and Economic Performance (Executive Order 13514), signed by President Obama in October of 2009, committed the federal government to lead by example in terms of energy sustainability and efficiency. This Order charged the government to reduce direct GHG emissions (fuels and building energy) by 28% and indirect GHG emissions (employee commuting and landfill waste) by 13% by 2020 (Obama, 2009). These measures were estimated to save the United States 101 million metric tons of CO_2 emissions, and 235 million barrels of oil (Obama, 2009). The Obama Administration also launched the Climate and Clean Air Coalition to Reduce Short-Lived Climate Pollutants to address the impact of methane, black carbon, and hydrofluorocarbons on public health, the environment, and world food productivity (Clinton, 2012). Both methane and hydrofluorocarbons were part of the original six GHGs EPA regarded as pollutants, and together with black carbon are responsible for nearly one-third of current global warming trends (Clinton, 2012). Partnering with the United States on this coalition are Bangladesh, Canada, Ghana, Mexico, Sweden, and the United Nations Environment Programme, each of which will produce a national action plan and policy priorities for reducing the impacts of these pollutants, including building capacity in developing countries, mobilizing funding, raising global awareness, fostering cooperation, and improving scientific understanding of pollution impacts and

mitigation strategies (Clinton, 2012). These programs relate to the Obama Administration's broader focus of an 'all-of-the-above' approach to energy development, in which Obama is pushing for safe and responsible development of all U.S. energy resources to provide sustainable, affordable energy to all Americans. While reducing GHG emissions clearly plays a large role in sustainability, it is but one aspect of the broad plan developed by the Obama Administration, which also includes developing clean coal, increasing natural gas production, reducing reliance on foreign oil by developing domestic oil reserves, reinvesting in nuclear energy technology, and advancing renewable energy development such as wind, solar, and biofuels.

Despite being traditionally conservative entities, U.S. military and intelligence agencies have recently openly embraced climate change impacts as a national security issue. In April of 2007, shortly after the Supreme Court decision in *Mass v EPA*, the Center for Naval Analysis released a report titled National Security and the Threat of Climate Change (CNA, 2007). This report was spearheaded by a board of 11 three- and four- star admirals and generals and highlighted the seriousness of climate change as a 'threat multiplier' that could exacerbate numerous existing national security issues, as well as create new threats and outlined mitigation and adaptation strategies for U.S. military forces (CNA, 2007). The report emphasized the complexity of climate change, saying "Unlike most conventional security threats that involve a single entity acting in specific ways and points in time, climate change has the potential to result in multiple chronic conditions, occurring globally within the same time frame" (CNA, 2007). That following year, the U.S. Army Strategic Studies Institute released a similar report outlining climate change impacts on water resources, infections diseases, political stability and conflict, and human migration (Pumphrey, 2008). The Navy in particular has been very active in this area, creating the Climate Change Task Force in May 2009, which published two Roadmap reports for

assessing impacts of climate change and building awareness and response strategies (TFCC, 2009, 2010). In 2011, the Navy also enlisted the aid of the National Research Council in outlining specific national security implications of climate change. The final report highlighted changes in geopolitical interactions and global navigation strategies in the Arctic, as well as increased need for global humanitarian assistance and disaster relief, and outlined research directives in these areas to increase response time and effectiveness and lay the groundwork for anticipating problems before they arise (NRC, 2011). The following year, the National Research Council released a similar report outlining climate change impacts on the intelligence and security communities as a whole (NRC, 2012). This report analyzed links between climate and socio-political factors including extreme weather events, vulnerability of individuals and societies, and the capacity of societies to respond to disruptions (NRC, 2012). The Department of Defense (DoD) often links climate change issues to energy security, which has been highlighted throughout the DoD's 2010 Quadrennial Defense Review (DoD, 2010) and the 2011 Operational Energy Strategy (DoD, 2011). In addition, numerous prominent national security officials, many conservative Republicans, have spoken out on the importance of assessing climate change threat impacts (Fitzsimmons, 2012). What is most interesting about these reports and statements is the lack of skepticism in climate science, particularly the lack of concern over scientific uncertainty. The 2007 CNA report states that "as military leaders, we know we cannot wait for certainty. Failing to act because a warning isn't precise enough is unacceptable." Military and intelligence agencies operate almost exclusively under conditions of high uncertainty and high risk, and perhaps for these reasons, are more receptive to incorporating climate change threats into their already complex risk analyses and threat defense strategies.

Another traditionally conservative body, the corporate world, has also recently become more involved in climate change policy. A number of major U.S. companies and environmental organizations created the U.S. Climate Action Partnership (USCAP) to advocate for national GHG emissions regulation. USCAP released a Blueprint for Legislative Action outlining potential cap-and-trade programs and international frameworks supported by the USCAP industries (USCAP, 2009). In 1998, the Center for Climate and Energy Solutions created the Business Environmental Leadership Council (BELC), which is now composed of nearly 40 companies spanning a range of industries with combined revenues of over $2 trillion (C2ES, 2012). In 2011, BELC member corporations publicly endorsed four guiding principles which accepted the scientific consensus of climate change and the risks of delayed action, encouraged businesses to establish strategies for GHG reduction, urged the U.S. to institute economy-wide, mandatory emissions reductions, and called for a balanced and effective global framework for climate change solutions (C2ES, 2012). Additionally, the Business for Innovative Climate and Energy Policy (BICEP) coalition represents 23 major U.S. companies and advocates for legislation that will guide the U.S. to a low-carbon economy, while still creating jobs and stimulating economic growth (BICEP, 2010). Specific policy goals supported by BICEP include a doubling of U.S. energy efficiency, 20% electricity from renewable sources by 2020 and 30% by 2030, shifting energy subsidies away from high-carbon fuels, restructuring energy prices to reflect environmental and economic costs, and providing support for developing countries to establish low-carbon energy strategies (BICEP, 2010). In support of these initiatives, the Environmental Defense Fund recently issued a Roadmap to Corporate GHG Programs that guides companies through the steps of measuring current emissions levels, setting targets, and designing strategies to achieve them (EDF, 2010). The roadmap is coupled with an Innovation

Exchange blog, which allows companies to share experiences and collaborate on GHG reduction programs (EDF, 2010) What all of these groups has in common is that each is a coalition of companies spanning a variety of industries and influences, perhaps none capable on their own of swaying policy, but together hoping to influence federal climate change legislation, and encourage other business through 'leading by example' with voluntary emissions reductions and energy efficiency actions.

The creation of these corporate organizations that support the scientific consensus on GHG emissions, and support federal policies for GHG regulation represent a significant shift from prior corporate stances opposing governmental regulation and attacking climate science. Historically, environmental regulations were met with protests of economic hardship and massive job loss from the corporate sector (Hecht, 2009). Industries consistently overestimated the costs of regulation and underestimated the benefits, including cost savings and technological innovation (Hecht, 2009). For example, when the 1990 Amendments to the CAA were passed, U.S. electricity companies claimed the cost of complying with the new regulations would exceed $4 billion per year, yet by 1996 these same companies were reportedly *saving* $150 million per year (Hecht, 2009). Despite repeated examples of such miscalculations (Hecht, 2009), industry continued to oppose additional regulations. For example, the Global Climate Coalition (GCC), a group of numerous major carbon emitting U.S. companies, was created in 1989 immediately following James Hansen's famous Congressional testimony on climate change. The goal of this group was expressly to oppose GHG regulations and encourage the federal government to avoid international agreements such as the Kyoto Protocol, and even testified before Congress concerning regulatory mechanisms (GCC, 2002). As early as 1997, member companies such as DuPont and BP voiced support for the scientific consensus and left the GCC for the pro-

regulation BELC (Brown, 2000). The GCC lost additional members after the release of the 2001 IPCC Assessment Report, including prominent companies as GM, Shell, Daimler Chrysler, and Texaco (Brown, 2000). These losses led the GCC to disband in 2002, though the website sites the reason for dissolution as a success of the organization's message, saying "the industry voice has served its purpose by contributing to a new national approach to global warming" (GCC, 2002). The GCC appears to have spoken too soon, as even leading carbon-intensive corporations now frequently support a stronger regulatory approach to GHG emissions.

As outlined above, the *Mass v EPA* Supreme Court decision marked the onset of a flurry of activity in all three branches of the federal government, as well as state-level governments, non-governmental organizations (NGOs), military and intelligence agencies, and industry. Though these agencies often acted under different (and sometimes conflicting) goals and operational structures, the overall picture these actors paint is of accepting climate science (though sometimes sluggishly or begrudgingly), incorporating potential impacts into risk analyses and strategic planning, and promoting other entities to do so as well. Though many of the regulatory structures and public responses outlined in this section are not necessarily the direct result of the *Mass v EPA* decision, this case arguably marked the beginning a new era in the U.S. approach to climate change issues.

Science and Policy

The legal discussions surrounding the authority of EPA to regulate CO_2 have largely ignored the scientific understanding of GHG emissions, and the broader scientific literature of global climate change. The Fabricant Memorandum addressed one important scientific point – the mixing behavior of CO_2 in the atmosphere. While NAAQS have proven effective against

numerous hazardous air pollutants, including ozone, carbon monoxide, and particulate matter (Fabricant, 2003), the unique nature of CO_2 to mix evenly throughout the earth's atmosphere, makes this regulatory approach fundamentally unsound scientifically. EPA regulations have circumvented this problem by avoiding the use of NAAQS for CO_2 regulation, focusing instead on regulation of emissions on a source-by-source basis, such as the NSPS. The Supreme Court dissenting opinion in *Mass v EPA* explicitly stated that the decision 'involves no judgment on whether global warming exists, what causes it, or the extent of the problem" (*Mass v EPA*, 2007). Additionally, the majority opinion upheld the argument of the petitioners not because scientific evidence had demonstrated the severity and immediacy of negative environmental impacts of climate change, but rather because the reasons EPA had given for choosing *not* to regulate were not allowed under the wording of the CAA (*Mass v EPA*, 2007). Specifically, EPA had cited political reasons for avoiding GHG regulation, including a potential impairment of negotiation strategies with other nations and the conflict GHG regulation would create between EPA and the Department of Transportation, including a non-integrated, piece-meal legislative environment. Interestingly, the majority opinion expressly stated that "we need not and do not reach the question whether on remand EPA must make an endangerment finding…only that EPA must ground its reasons for action or inaction in the statute" (*Mass v EPA*, 2007). This wording meant that EPA was required to base its course of action on the body of scientific literature on climate change impacts of GHGs and not policy considerations, but that the Supreme Court did not purport to know what course of action the scientific literature would support.

The Supreme Court decision highlights an important and pervasive problem in science policy – the inclusion of political, economic, and corporate considerations as deciding factors. Such considerations are especially evident in climate change litigation and legislation, where

science (sometimes factually presented and other times misrepresented) is frequently used as a tool to forward viewpoints on all sides. In 2005, the House of Representatives Committee on Oversight and Government Reform released a report on political interference in climate change science, which concluded "the Bush Administration has engaged in a systematic effort to manipulate climate change science and mislead policymakers and the public about the dangers of global warming" (COGR, 2007). The report highlighted numerous instances of censoring of scientists' Congressional testimony and conversations with the media and extensive editing of government scientific reports on climate change issues (COGR, 2007).

Legislators also promoted the economic concerns touted by industry in the early 2000s. For example, President Bush highlighted the economic costs of environmental regulation in his 2002 *Economic Report of the President* and downplayed environmental concerns, at one point stating "Now that most of the largest and most glaring environmental problems have been tackled, the gains to be expected from further measures have become less obvious and more contentious" (Bush *et al.*, 2002). Considering the current scientific consensus on climate change impacts, the thought that our 'most glaring environmental problems have been tackled' is almost laughable, yet such comments can have a major impact on political viewpoints and federal environmental policy. This effect is evident in the shift towards support of environmental regulation described in the previous section. Since 2008, President Obama and his Administration have frequently voiced support for climate change science and linking regulatory mechanisms with economic concerns to tackle multiple problems at once (Daynes & Sussman, 2010). Though the 2007 economic recession forced some of Obama's environmental platforms to be postponed, recent improvements in economic conditions have opened opportunities for addressing this issue, and the response has been widespread, as outlined in the previous section.

Unfortunately, presidential statements are not the only relevant perspective. Polarization between Republicans and Democrats in Congress is currently the highest it has been since 1885 and has been continually increasing since the 1970s (Fig. 4.1, McCarty *et al.,* 2011). Consequently, though the Obama Administration (and even Democratic members of Congress) may support climate change legislation, Republican politicians may still aggressively oppose it. For example, the previously mentioned AEI hearings in E&P frequently focused on economic hardship and job losses in the coal industry, stating that EPA regulations would result in the extinction of American coal plants (E&P, 2012).

In the face of such interference and resistance, it is not surprising that many scientists are at best reluctant to be involved in politically charged scientific discussions and that communication between scientists and policy makers is often minimal. Many scientists have taken the 'loading dock' approach whereby information is simply released (i.e. published in peer-reviewed journals) and may or may not be 'picked up' by decision makers (Cash *et al*, 2006). In such situations, a lack of readily available and usable scientific knowledge, and competing interests for policy makers can lead to non-existent or ineffective legislation. It is increasingly important for robust scientific information to be available to and usable by policy makers. Effective science policy relies on iterative communication between knowledge producers (scientists) and consumers (policy makers), requiring both sides to actively 'own the problem' of producing and communicating usable science and translating scientific knowledge into effective legislation (Lemos & Morehouse, 2005; Dilling & Lemos, 2011). Improvements in this relationship can be made on both sides; for example, by improving scientists' understanding of the context of scientific information in political decision making, and improving policy

makers understanding of the scientific method and intricacies of predictability, uncertainty, and consensus.

Unfortunately, legislative action and constituent pressure lag increasingly far behind the need for decisive action. Previous climate change regulation discussions (such as the 2009 Copenhagen Accord) had revolved around a 2° C global increase in mean temperature as the threshold between 'acceptable' and 'dangerous' climate change impacts (Copenhagen Accord, 2009). Recent analyses show that there is now little to no possibility of maintaining a global temperature rise blow the 2° C threshold, and that this level may actually represent the boundary between 'dangerous' and 'extremely dangerous' climate change (Anderson & Bows, 2011). Much of the political debate and statements from high-level officials, however, maintain the outdated goals of a less severe, and still achievable 2° C cap (Anderson & Bows, 2011), illustrating the need for increased dialogue between scientists on the cutting edge of climate change research and legislators in the process of formulating national and international regulatory frameworks.

Science and the Public

The previous section described the complex relationship between scientists and policymakers. It is not to be forgotten, however, that the general public also play a major role in the science policy realm. Policymakers may be unlikely to propose or pass legislation that is unpopular with or deemed unnecessary by their constituents. Consequently, a large body of research has delved into the public understanding of climate change issues (Bord *et al.*, 2000; Stamm *et al.*, 2000; Zia & Todd, 2010; Kim, 2011), trust in scientists and scientific knowledge (Cooper, 2011; McCright & Dunlap, 2011; Gauchat, 2012), and the role of the media in

informing the public on science (Stamm *et al.* 2000; Wilson, 2000; Zehr, 2000). For example, in 1992 Sage Publications created a journal titled Public Understanding of Science, which publishes peer-reviewed literature on science education, popular representations of science, and the relationship between science and the media. The fact that such a journal even exists, and the high ranking of the journal (Thomson Reuters, 2012) are a testament to the importance of the public's role in scientific issues. Similarly, the journal *Frontiers in Ecology* published a special issue focusing on effective communication of science in environmental controversies in August 2010. This journal has published only ten special issues since its inception in 2003, illustrating the importance of communicating controversial science to the public. This section will discuss the important roles that societal understanding of science, communication between scientists and the public, and the terminology used in policymaking play in effective science policy.

Public surveys administered by organizations such as Gallup and the Pew Research Center reflect interesting trends on public attitudes towards scientists and knowledge on scientific issues. Environmental issues have been included on surveys for nearly 40 years; however, questions relating directly to global warming or climate change have been included only since 1986 and the nearly 300 surveys conducted since this time show widely varying question structure and frequency of surveying making it difficult to construct a clear long-term picture of public opinion (Brulle *et al.*, 2012). Despite these differences, many of these surveys reflect significant recent declines in the public's acceptance that climate change exists, is human-caused, and represents a serious threat (Pew Center, 2008, 2009; Gallup, 2009, 2010; Weber & Stern, 2011; Leiserowitz *et al.*, in press; McCright & Dunlap, 2012; Scruggs & Benegal, 2012), which as been termed by some a 'crisis of confidence' in climate science (Scruggs & Benegal, 2012). These declines have been attributed to economic trends (Brulle *et al.*, 2012; Scruggs &

Benegal, 2012), the influence of political elites (Brulle *et al.*, 2012; McCright & Dunlap, 2011), scientific scandals such as 'climategate' (Leiserowitz *et al.*, 2012), media coverage of climate change issues (Brulle *et al.*, 2012), and anomalous short-term weather patterns that appear to contradict climate change predictions (Li *et al.* 2011). In order to elucidate the relative impacts of each of these potential drivers, Brulle *et al.* (2012) combined the results of 74 separate surveys conducted from 2002 to 2010 into a broad-spectrum Climate Change Threat Index (CCTI) that reflects the overall 'policy mood' of the public in regards to climate change issues. Brulle *et al.* (2012) then developed a series of models testing the separate and combined effects of numerous drivers. They found that the strongest overall predictor of public opinion was the actions of political elites, specifically public pro-climate change policy statements by Congressional Democrats, and anti-environment voting records of Congressional Republicans (Brulle *et al.*, 2012). Also significantly affecting public opinion were the release of high-profile scientific assessment reports, such as those by the IPCC, popular science articles (i.e. not peer-reviewed literature) and other media coverage of climate change, U.S. gross domestic product (GDP), unemployment rates, and military casualties in Iraq and Afghanistan (Brulle *et al.*, 2012). Not significantly affecting public opinion were short-term weather events and peer-reviewed science publications, which likely do not reach the public audience (Brulle *et al.*, 2012).

Such an aggregate method of measuring climate change perceptions may miss important differences by demographics or by specific climate change issues. For instance, Brulle *et al.* (2012) showed that, in aggregate, short-term weather events did not affect overall public opinion; however, Li *et al.* (2011) showed that daily temperatures, and the perception that they were above or below average for that region, significantly affected respondents' belief in and concern over global warming. Similarly, though overall public knowledge and acceptance of climate

change may be low, recent surveys in Alaska and Florida, where impacts of climate change have been especially pronounced, reflect higher levels of public concern and willingness to take political action in these regions (Arctic Climate Impact Assessment, 2004; Leiserowitz & Broad, 2008). Interestingly, while Brulle *et al.* (2012) report a steady rise in CCTI from 2005 to 2007, the Pew Center for Research for the People and the Press (2012) report a significant drop in Americans indicating belief that the earth is warming across the same time period (Fig. 4.2). The CCTI then drops steadily from 2007 to 2010 (aside from a spike in 2009), while the number of Americans reporting belief in warming reaches a minimum in 2009, and has risen steadily since then (Fig. 4.2; Brulle *et al.*, 2012; Pew Center for Research, 2012). The reason for these different patterns is unclear, but likely relates to the specificity of the Pew Center question as opposed to the broad aggregate measure of the Brulle *et al.* (2012) study.

Overall, these surveys indicate that external issues (such as the economy, the opinions of political elites) not only affect the public's perception of climate change as a threat, but also the actual belief that climate change is even happening (Scruggs & Benegal, 2012). This is a curious effect, as changes in external factors should not alter a person's perception of scientific evidence, yet that appears to be exactly what occurs. Another peculiar trend is the public support of climate change legislation compared to public perceptions of climate change evidence. While only 36% of Americans reportedly believed there was solid evidence for human-caused global warming, 50% of Americans favored a national cap-and-trade system and 88% supported setting national standards for carbon emissions either on our own, or through international agreements (Pew Center for Research, 2009). These counter intuitive trends and variations across study scale and question structure illustrate the complexity and dynamism of the public's viewpoints on climate change, and the difficultly in accurately representing these trends through time.

Of particularly relevance to the *Mass v EPA* decision are the surveys analyzing the public's confusion of climate change issues with air pollution. Specifically, many Americans cite chloroflourocarbons (CFCs, Stamm *et al.*, 2000; Weber & Stern, 2011) or aerosols and insecticides (Bord *et al.*, 2000) as important causes of global warming, and recommend regulating air pollutants to reduce warming (Reynolds *et al.*, 2010). Such confusion was especially prominent in the early 1990s, when ozone depletion was a major environmental problem with which the public was quite familiar (Kempton, 1991). The subsequent 20 years of research and public outreach have helped to lessen the prevalence of this confusion, but significant misconceptions remain (Bord *et al.*, 2000; Stamm *et al.*, 2000; Reynolds *et al.*, 2010). In a political context, such misconceptions may not be problematic, as even inaccurate understanding of climate change causes can positively impact the belief that climate change is occurring and the willingness to take action to prevent it (Bord *et al.*, 2000). What remains troublesome about this confusion is that most air pollutants are very short-lived in the atmosphere, and reduction of their emissions rapidly results in reduced pollutant levels and reduced negative impacts of pollution. GHGs on the other hand, have much longer atmospheric lifetimes, and can continue to impact climate for decades or even centuries after they are emitted (Solomon *et al.*, 2009). The confounding of climate change with air pollution may then lead laypersons to underestimate the negative consequences of delayed action, and/or to support regulatory measures that may not actually address climate change causes. Labeling of CO_2 and other GHGs as air pollutants may help alleviate some confusion by highlighting the importance of CO_2 as a cause of climate change, but may also contribute to confusion by accidentally promoting the misconception of rapid and straightforward reductions in pollutant levels.

Despite the recent volatility in public understanding and acceptance of climate change causes and consequences, there are some promising trends in public surveys. For example, 'trust in science' has remained fairly constant over the last 35 years in liberals and moderates (though conservatives show a slow but consistent decline since 1985, Fig 4.3; Gauchat, 2012). Additionally, scientists are still considered the most trusted source of information on climate change (Leiserowitz *et al.*, 2012), and though many Americans (32%; Gallup, 2012) still assume a high level of disagreement amongst scientists, this belief has been decreasing over recent years, and since 2000, a majority of Americans have believed that most scientists believe global warming is occurring (Gallup, 2012). Depending on the survey and question structure, it appears public skepticism may have peaked in 2009 or 2010, and since then has begun to decline (Gallup, 2012; Pew Center for Research, 2012), which sets a hopeful tone for future trends in public understanding and acceptance of climate change, and support for related federal policy.

It is also worth asking if public perspectives truly drive climate change policy. While the lack of universal public acceptance of climate change causes and consequences may be irksome to scientists, we must ask to what degree do public attitudes towards climate change and GHG regulatory policies actually translate into legislation and policy frameworks? The answer appears to be very little. First, as previously mentioned, Americans who remain skeptical of climate change may still support climate change legislation (Pew Center for Research, 2009). Second, despite low public perception of climate change threats throughout the mid-2000s, traditionally conservative institutions such as the corporate world and the U.S. military publicly supported climate change initiatives (see previous section for details). Amongst these groups, security and risk preparedness seem to outweigh public ambivalence and these groups have demonstrated their willingness to proceed ahead of public support (Nagel, 2011). Third, public opinion is

almost completely irrelevant in climate change litigation. Judges on federal and state supreme courts, as well as many lower courts, are appointed by the president or the state governor, not elected by the people. Consequently, pleasing constituents in order to ensure reelection is not a relevant consideration in court proceedings. As evidenced by the litigation of EPA regulations following the *Mass v EPA* case, so long as environmental regulations are within the legal statutes, lawsuits may be filed by disgruntled public entities, but are unlikely to be supported by judicial review. It, therefore, appears that a strong public push for climate change legislation is not a prerequisite for effective corporate or federal policy.

Communicating Science

In several places, this article has highlighted the need for increased communication between scientists and the public and political arenas. Recently, a number of venues have been created through which scientists hope to better communicate with both of these groups. This section will discuss current initiatives to foster communication about science (particularly climate change issues) between scientists, policymakers, and the general public and will highlight strategies for future communication venues.

ScienceDebate.org is a nonpartisan non-profit organization that was created by independent citizens and cosponsored by scientific organizations such as the American Association for the Advancement of Science (AAAS, publishers of the top-rated, peer-reviewed journal *Science*), the National Academies, and economic organizations such as the Council on Competitiveness. ScienceDebate contacts candidates, such as Barack Obama and Mitt Romney during the 2012 presidential race, with lists of science based questions, and posts their responses online for side-by-side comparison by voters. ScienceDebate questions relate to innovation and

the economy, climate change, scientific research budgets, biosecurity, STEM (science, technology, engineering, mathematics) education, energy policy, food security, safe water resources (including drinking water and ocean health), cybersecurity, natural resources, space exploration, and public health policy. Candidates' responses to such questions allow voters to make informed science policy choices, especially when science topics are not prominent issues in the televised debates or candidate speeches. ScienceDebate also offers educational seminars for the public and policymakers, and science education workshops for the media to enhance information exchange between all parties.

Skeptical Science is an online community of 25 contributors, including climatologists, geologists, chemists, physicists, computer scientists, and environmentalists from throughout the world. This community encourages scientific skepticism by challenging the public to review scientific information on climate change and 'get skeptical about global warming skepticism.' SkepticalScience.com organizes scientific responses to the majority of climate change denial arguments, providing evidence at a variety of complexity levels to match any reader's knowledge base. Responses are available in nearly 20 languages, and include concise overviews, detailed explanations, graphics of relevant data with links to original sources, and interactive tools for sharing information on social media.

The National Science Foundation recently sponsored the creation of an online climate change resource module geared towards educators, called Climate Adaptation Mitigation E-Learning (CAMEL). CAMEL is a free, comprehensive, interdisciplinary, and multi-media resource for K-12 and college level educators to access natural and social science research relating to all aspects of climate change, which are grouped into causes, consequences, solutions, and actions. CAMEL supplies lab experiments, homework assignments, field exercises,

podcasts, simulations, and games, as well as actual IPCC-based data sets and real-life case studies for teachers to engage students of all levels in climate change issues. As teachers utilize various CAMEL resources, they can rate each module, and provide online comments to aid other educators in choosing the best resources for their classroom needs.

Unfortunately, these strategies subscribe to a number of science education fallacies. First, all of theses initiatives take the 'loading dock' approach, whereby information is 'dropped off' and it is assumed anyone interested will come 'pick it up' (Cash *et al.,* 2006). Since climate change topics are highly politically charged, selective exposure (i.e. the tendency to seek out information that supports previously-held opinions) is common, particularly among people who believe climate change to be naturally caused and not a result of human activities (Kim, 2011). Consequently, passive 'loading dock' approaches to information dissemination are unlikely to reach climate deniers or impact their decisions. These strategies also cater to the idea that public misunderstandings of science issues are related only to gaps in knowledge and that transferring information from experts to lay persons will fill this gap. This approach is termed the 'deficit perspective' and assumes that information only needs to travel from scientist to layperson in order to reduce the laypersons knowledge deficit, with minimal need or opportunity for dialogue between both parties or for engagement of laypersons in the scientific process (Cooper, 2011). This perspective assumes that persons with high science literacy will be more likely to support the scientific consensus on climate change, and so increasing public science literacy will increase public acceptance of science. This assumed correlation has been shown to be false, particularly amongst conservatives, and particularly in regards to climate change issues (McCright & Dunlap, 2011; Zia & Todd, 2010). Much research has been conducted on the mental models Americans use to understand climate change (ex. Weber & Stern, 2011), and these studies reflect the

inadequacy of the deficit model educational approach, highlighting the fact that members of the public who do not understand climate change "are not a blank slate…so the needed educational process is not one of adding to knowledge but one of inducing conceptual change" (Weber & Stern, 2011). Though far more difficult to structure and fund, more active and engaging opportunities are needed, not just to educate the general public (i.e. fill the knowledge gap), but also to build a foundation of engagement in science, and promote conceptual change in the American public.

A number of strategies have been suggested for achieving more effective communication between scientists and the public and policymakers. These strategies often promote reframing scientific issues in a way that resonates more strongly with the audience. 'Framing' relates to how a topic is conceptualized, meaning what aspects of the topic are emphasized, and helps individuals focus on the relevance of a given topic to their everyday lives (Groffman *et al.* 2010). For example, climate change issues can be reframed as economic issues, where investment in clean technology can produce new jobs; as a public health issue, where climate change may impact prevalence of infectious diseases or heat stroke; or even as an environmental stewardship issue, where environment-friendly actions are made for moral or ethical reasons, rather than scientific ones (Groffman *et al.*, 2010). Zia and Todd (2010) suggest reframing climate change from a 'butter' issue to a 'guns' issue. 'Butter' issues include education, health, and social welfare and are generally associated with liberal ideology, while 'guns' issues focus on defense and security and are more associated with conservative ideology (Zia & Todd, 2010). Since conservatives are generally less supportive of climate change regulatory policies, reframing climate change as a 'guns' issue could help reach this reluctant audience. 'Guns' issues in climate change include security issues, like energy independence and risk management, and, Zia

and Todd (2010) argue, could be publicized similarly to the color-coded terror threat levels. Economic impacts can also be viewed as 'guns' issues through increasing energy efficiency (i.e. cutting spending) and stabilizing economic responses to uncertainty (Zia & Todd, 2010). The U.S. military has already begun reframing climate change as a 'guns' issue in many of these ways (DoD, 2010, 2011). The recent economic crisis undermined the public's confidence in short-term economic policies, and laid the foundation for public support of longer-term policies focusing on investments in future technologies, creating an opportunity for effective economic reframing of climate change legislation (Zia & Todd, 2010). Also, since conservatives tend to be more religious than liberals, climate change could be framed as a moral issue of compassion and justice, whereby mitigation and adaptation strategies alleviate suffering of communities and habitats (both human and animal) at risk from climate change impacts (Zia & Todd, 2010). Scientists may well be reluctant to contextualize science in a religious framework; regardless, this strategy may prove effective for reaching a wider political and social audience. Another frequently highlighted frame is that of risk management, where climate change is communicated similarly to other everyday risks that Americans face, such as disease or automobile accidents (Weber & Stern, 2011). Americans are used to making decisions to avoid exposure to or reduce the severity of these sorts of risks, and may be able to fit climate change impacts into a similar mental framework (Weber & Stern, 2011). The relative merits and pitfalls of each of these framing approaches still need careful investigation, and it is likely that some frames will work in certain contexts and not others. It may behoove scientists to become comfortable discussing climate change in such contexts in order to engage members of the public and political worlds more effectively.

Kansas recently put this strategy to the test through the creation of the Climate Energy Project (CEP) and the initiation of the Take Charge Challenge (Larsen, 2012). Frustrated with the previously-mentioned battle over the Sunflower Electric coal plant proposal in Holcomb, KS, Wes Jackson (founder of the Land Institute in Salina, KS) and his daughter-in-law, Nancy Jackson created CEP, a non-profit organization dedicated to reducing GHG emissions throughout the Heartland (CEP, 2013). CEP created the Take Charge Challenge in 2009 which originally focused on six communities throughout Kansas (Larsen, 2012). The Challenge was structured as a competition between the communities, providing a $20,000 prize (dedicated to an energy upgrade project) for the community which reduced energy use the most (Larsen, 2012). CEP set up informational events like energy efficiency-themed ice cream socials, holiday tree lightings (with lower energy LED bulbs), neighborhood block parties, and attended county fairs, senior centers, and back to school nights to spread information (CEP, 2010). CEP restructured the climate change discussion in these communities, by focusing on classic Midwestern values of patriotism, thrift, and local pride (Larsen, 2012). Jackson argues this approach is not a way to 'spin' climate change. "It's about genuinely listening to our audience and then engaging on their terms," said Jackson in one interview. "It's not about 'framing.' It's about honoring a different way of knowing" (Larsen, 2012). Whatever you call it, the Take Charge Challenge has been impressively successful. The first round of the Challenge saved an estimated $412,000 in energy costs for Kansas residents and prevented the emission of 6 million pounds of CO_2 (CEP, 2010). The impressive public engagement and effectiveness of the first round spurred a second round incorporating 16 Kansas communities and four prizes of $100,000 each (Larson, 2012). In total, the communities involved in the second Take Charge Challenge saved 110.2 billion british thermal units (BTUs) of gas and electricity, over 19,000 barrels of oil, and 22 million pounds of

CO_2 emissions, reducing residents energy costs by over $2.3 million annually (CEP, 2011). A third round is currently in the planning stages and is aimed at setting the competition amongst corporations from eight cities throughout Kansas, Missouri, Oklahoma, and Nebraska (Larson, 2012). CEP has effectively restructured the conversation to engage traditionally conservative heartland communities regardless of their opinions about climate change. Says one resident, Mark Richardson of Hutchinson, KS, "[Debating global warming is] a waste of time and distracts from the economic and tax policy discussion. Dollars are a lot easier for people like me to understand than CO_2 levels, and the conversation doesn't have to involve the entire planet." CEP, therefore, has overwhelmingly illustrated the power of communicating with the public 'on their terms' and engaging citizens in the climate change and science policy debate in ways that are meaningful to them.

The Take Charge Challenge is a classic example of communication through the model of public engagement in science (PES), which focuses on mutual learning between citizens, scientists, and policy makers (Groffman *et al.*, 2010). This model differs in many ways from the deficit model (Table 4.1) in that the public is engaged in a dialogue with scientists, which increases trust between both groups and recognizes the importance of societal values and political context of scientific information (Groffman *et al.*, 2010). The effectiveness of the PES model requires training of scientists to engage with the public in this manner, including development of communication skills for graduate and undergraduate science students, interdisciplinary research and degree programs to foster communication and collaboration amongst fields, and professional development programs for scientists at all levels to promote effective dialogue between scientists and the public, policymakers, and the media. Increasing the opportunities for such training will aid the scientific community in supporting grass-roots

endeavors like the Take Charge Challenge, as well as in advising policymakers on effective legislation.

Communicating science to non-scientists is a highly complex endeavor, and there are many levels at which improvements in the science policy relationship can be made. For example, environmental policy issues are often framed almost entirely as scientific problems, while these issues nearly always involve concerns outside of science as well (Pouyat *et al.*, 2010). It is important in science policy discussions to separate what are science questions from what are policy questions; for example, science can address the amount and types of risk posed by an environmental contaminant, but only policy can address how much environmental damage from that contaminant is 'acceptable' before regulatory measures must be put in place (Pouyat *et al.*, 2010). In a climate change context, science could outline mitigation or adaptation strategies (ex. reduce GHG emissions) though legislators must decide how to pursue these strategies (ex. cap and trade, carbon tax, renewable energy). Past successful examples of science policy interaction have illustrated the importance of continuous contact between scientists and policy makers at all stages of the research and legislative processes (Pouyat *et al.*, 2010). Too often, scientists are only included at the end stages of legislative development, to approve already-developed regulatory measures. The development of long-term, mutually beneficial relationships between scientific and political agencies (as well as between scientists and politicians) may help infuse relevant scientific information into policy deliberations throughout the numerous stages of policy development (Pouyat *et al.*, 2010).

One way to increase the quantity and quality of communication is through interface organizations between science and policy. Interface organizations can function in a number of ways, including convening scientists with relevant experience for a specific policy question,

building long-term collaborations between scientists and knowledge users (such as policymakers), facilitating the 'translation' of scientific research into more user-friendly terms, and building reward systems for information sharing and educating both sides on the learning and communication style of the other (Osmond *et al.*, 2010). An example of one such interface organization is the Hubbard Brook *Science Links* program. The purpose of this program is to identify critical science policy questions, collect and analyze existing data, and evaluate the effectiveness of policy options for addressing the issue (HBRF, 2012). Findings are then reported to relevant policy makers, the national media, and made available online for the general public, and have included topics such as acid rain, nitrogen pollution, mercury, and carbon dioxide emissions (HBRF, 2012). The Carbon and Communities report, released in 2011, convened scientists from numerous U.S. universities, as well as national laboratories and environmental organizations (Fahey *et al.*, 2011). The report included detailed background information on the global carbon cycle and the role of CO_2, analyses on carbon sources and sinks in the U.S. Northeast (the region of focus for the report), then outlined mitigation options such as energy efficiency, wind power, solar water heating, geothermal heating, and rooftop solar power generation (Fahey *et al.*, 2011). The report tailored recommendations by county, taking into consideration each county's current carbon emissions and the cost of different mitigation strategies in that region (Fahey *et al.*, 2011). The *Science Links* program illustrates one way in which rigorous, peer-reviewed literature can be disseminated to a wider audience and made relevant and effective in a policy context. There are many styles and structures of interface organizations, which appear to be most effective when focused on a single issue in a clearly described region, and when they are able to go beyond merely informing, but also engaging diverse audiences (Osmond *et al.*, 2010).

Another important, but controversial, component of communicating science to non-scientists is through advocacy. Scientists are generally incredibly hesitant to become active in environmental advocacy, and often prefer to 'let the facts speak for themselves.' Some have argued that scientists should almost exclusively use non-persuasive communication and avoid becoming 'peddlers rather than arbiters of truth' (Fischhoff, 2007). Others argue that scientists can be both objective researchers and passionate citizens and can make effective advocates for science policy (Meyer *et al.*, 2010). Those in favor of scientists as policy advocates argue that, as experts, scientists are well suited to present information in a factual and persuasive way, and highlight the obligation some scientists have felt towards making an impact in policy (Meyer *et al.* 2010). So long as scientists make it clear when they are speaking on facts as opposed to speaking on their personal values, advocacy should not undermine public trust in the objectivity of science or the reputation of the scientist him/herself (Meyer *et al.*, 2010). Opponents argue that scientists should focus only on the facts, and let other fields (decision science, social science) address public values, perceptions, and goals (Fischhoff, 2007). Though arguments have long been made on both sides, it is unlikely that many scientists will risk the public debate and professional ostracism that can accompany open political advocacy until or unless such actions are sanctioned by their peers or somehow incentivized within the scientific career path.

Conclusion

The *Mass v EPA* court case marked the end to a decade-long debate over the classification of CO_2 as an air pollutant, and the subsequent regulation of CO_2 by EPA under the CAA. In a much broader sense, however, the *Mass v EPA* case marked the beginning of a new era of U.S. climate change legislation and policy across numerous political, public, and corporate

entities. Even five years later, under a new president, with a different make-up of Congresspersons, with new governors and state congresses, we see the beginnings of a sustained commitment to climate change issues, to reducing GHG emissions, and to protecting human health and welfare (including the economy) through our mitigation and adaptation strategies. We see the possibility of new collaborations between industry and government, between citizens and scientists, and (if Congressional polarization begins to ebb) hopefully between conservatives and liberals. This cooperation must continue if we are to address the causes and consequences of climate change in a timely and effective manner.

Chapter 4 Tables and Figures

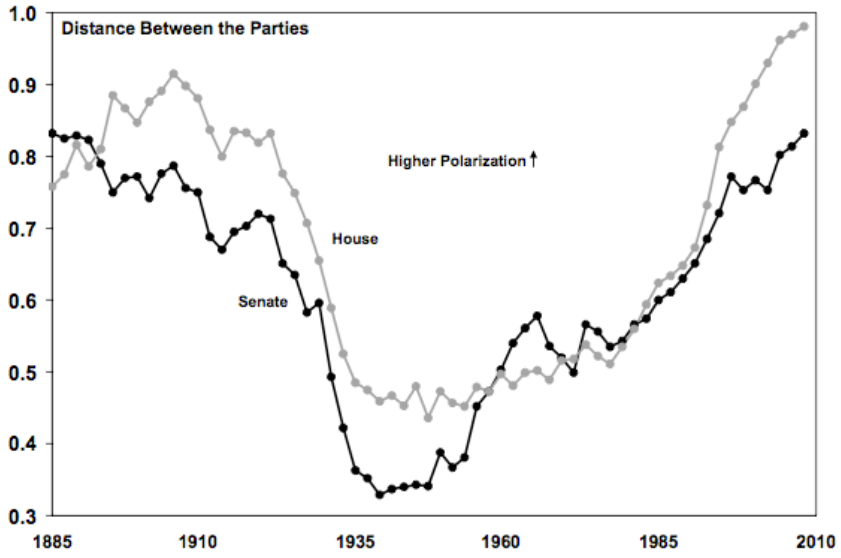

Figure 4.1 Polarization between Republicans and Democrats in each house of Congress, derived from voting patterns and based on the relative divergence in the average position of each party. Taken from McCarty *et al.* 2011.

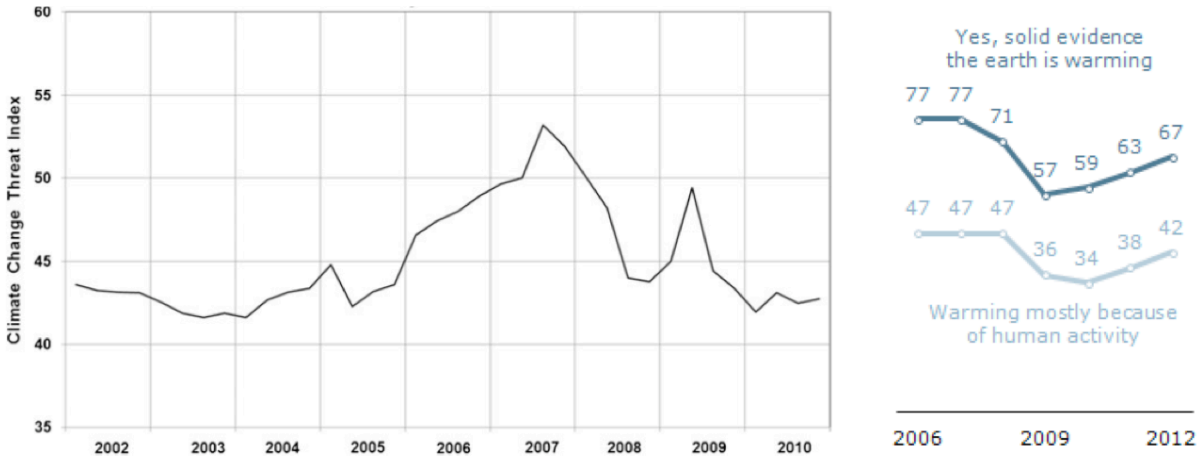

Figure 4.2 Differences in trends of the CCTI (left) and belief in global warming (right). CCTI trends taken from Brulle *et al.* 2012, belief in global warming trends taken from Pew Center for Research, 2012.

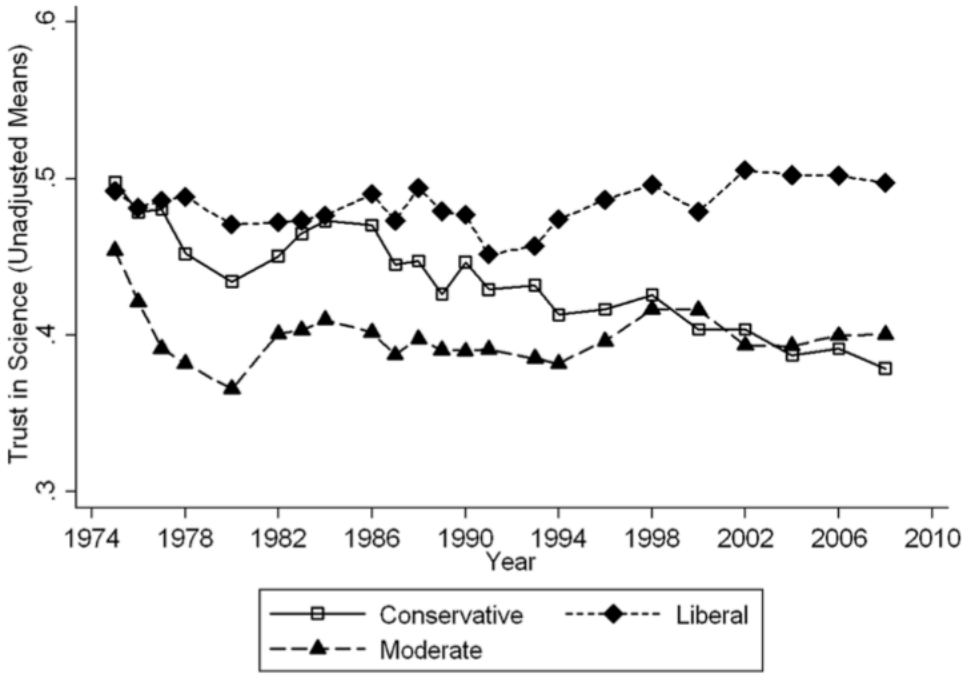

Figure 4.3 Unadjusted means of public trust in science for each survey year by political ideology. Figure shows three-year moving averages for each group, which smooth the pattern over time. Taken from Gauchat, 2012.

Table 4.1 A comparison of selected aspects of the deficit and public engagement models

Aspect	*Deficit model*	*Public engagement model*
Major influence(s) on public beliefs and decisions	Science literacy or the lack thereof	Values, trust, identity, and social networks
Proposed solution to societal inaction	To improve science literacy (ie to fill in the "deficit" in the public's technical understanding of an environmental problem)	To connect an environmental problem to public values while building trust and empowering public participation
Communication is a process of…	…transmission, which means "popularizing" and "simplifying" technical information that flows from experts to the public	…dialogue and the two-way exchange of perspectives; both the public and experts learn from this process
The definition of "reaching the public"	Increasing the amount and technical accuracy of science news coverage, focusing on traditional outlets such as the newspaper science beat, popular science magazines and books, or public television programming	Reframing a complex issue around relevant and familiar dimensions; engaging in local community forums and dialogue; partnering with opinion leaders and other societal groups; and complementing traditional science coverage with novel entertainment genres and social-media initiatives
Scientists and their organizations…	…are under attack in society; any communication failures are blamed on public ignorance, the media, or "politicization" and "anti-science"	…hold almost unrivaled trust, authority, and respect in society; scientists need to use this communication capital effectively and wisely, otherwise scientists share some of the blame for communication failures
The ultimate goal	To improve science literacy – once the public is brought up to speed on the science, they will view issues and decisions as scientists do, controversies will go away, and progress will occur in dealing with environmental problems	To motivate, enable, and empower the public to make decisions about environmental problems – yet, no matter how accurately communicated and understood the science, public decisions cannot be separated from values, political context, and necessary tradeoffs between costs, benefits, and risks

Taken from Groffman *et al.*, 2010

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Conclusion

During the Last Glacial Maximum (LGM; 18,000–20,000 yr ago) and previous glacial periods, atmospheric [CO_2] dropped to 180–190 ppm, which is among the lowest concentrations that occurred during the evolution of land plants. Modern atmospheric [CO_2] is more than double that of the LGM and 45% higher than pre-industrial levels. Since CO_2 is the carbon source for photosynthesis, lower carbon availability during glacial periods likely had a major impact on plant productivity and evolution. Impacts of low [CO_2] transcend several scales, ranging from physiological effects on individual plants to changes in ecosystem functioning, and may have even influenced the development of early human cultures via the timing of agriculture. In a current environment of increasing atmospheric [CO_2] levels, and considering predictions for continued future increases, it may seem odd to be considering plant responses to low [CO_2]. It is important, however, to develop a strong foundation in understanding plant responses to low [CO_2] as this knowledge is critical for predicting the implications of rising [CO_2], explaining plant evolutionary patterns over geologic time scales, and estimating past and future levels of net primary productivity. In this way, an increased understanding of plant responses to low [CO_2] anchors modern and future plant responses to the geologic past by determining how natural global change factors in the past may continue to influence plant responses to future anthropogenic changes.

In order to assess plant responses to low [CO_2] over geologic time scales, preserved glacial wood material was analyzed and compared to modern trees from the same regions. Two separate systems, *Juniperus sp.* and *Agathis australis*, were sampled to provide a broader perspective. Glacial *Juniperus* specimens spanning the last 50,000 years were obtained from the La Brea tar pits in Los Angeles, CA. Glacial *Agathis* specimens, 50,000+ years old, were

obtained from peat bogs in the Northland region of North Island, New Zealand. In both cases, wood material is well preserved in its original organic state, allowing for annual ring width and isotopic analysis. Modern trees were also sampled from both locations. Modern *Juniperus* cores were taken from trees currently growing in the San Bernardino Mountains. Government regulations prevent sampling of living *Agathis*, so wood remnants spanning the last 3,000 years were salvaged from construction sites in Northland. In both systems, ring width and carbon isotope analysis was performed to compare physiological responses to changes in [CO_2] and environmental factors since the last glacial period. Carbon isotopic signatures were used to calculate c_i/c_a (the ratio of internal CO_2 availability to that of the atmosphere) and c_i. Oxygen isotope analysis was also performed on the *Juniperus* system to analyze responses to anomalous climatic events, namely the El Niño Southern Oscillation.

Both *Juniperus* and *Agathis* showed constant mean c_i/c_a between the last glacial period and modern times resulting in extremely low glacial c_i values. On average, mean c_i was approximately half the modern c_i levels in both species. When looking at all individual rings, glacial and modern c_i levels were almost completely non-overlapping, meaning glacial trees experienced c_i values that are so low as to be outside the range of modern experience. These results suggest severe carbon limitations in glacial trees, which could have impacted primary productivity and annual growth patterns.

Interestingly, however, glacial *Juniperus* and *Agathis* trees show similar mean and maximum ring width as modern trees. Despite having less than half the available carbon, glacial plants were able to maintain nearly identical growth patterns as their modern counterparts. Growth-c_i relationships were non-significant for the majority of all trees, meaning that over the lifetime of a single individual, increased c_i did not result in increased annual growth, even in

glacial conditions of severely reduced [CO_2]. We attribute this lack of CO_2 fertilization on tree growth to environmental constraints specific to each region, and constraints resulting from adaptations to 10-14 million years of low CO_2 conditions.

Oxygen isotope analysis was performed on glacial and modern *Juniperus* to reconstruct El Niño impacts in southern California over the last glacial period. The El Niño Southern Oscillation (ENSO) is currently the most significant source of global climatic variability, though determining paleo-ENSO patterns is plagued by uncertainties in model parameters as well as varying results from proxy-based reconstructions. In arid regions such as the southwestern United States, ENSO-induced increases in growing season temperature and precipitation register as wider annual rings. Oxygen isotopic content of ring alpha-cellulose reflects dynamics of water availability during growth and can also be used to reconstruct atmospheric conditions. A *Juniperus* ENSO prediction model developed by Nippert *et al.* in 2010 was applied to modern *Juniperus* at high elevations that are growing in climates similar to lowland conditions during the last glacial period. Interestingly, we find that *Juniperus* physiological responses to El Niño conditions at higher elevations greatly differ from those at lowland sites used to develop the model. Under less water-limited growing conditions, *Juniperus* does not respond as strongly or as predictably to ENSO-induced changes in temperature and precipitation. This result suggests the same could be true for glacial trees, which could confound proxy-based results in this region. A deeper understanding of the climate-physiology relationship of a species under different environmental conditions is required before a reliable paleo-proxy can be developed.

This research advances our understanding of plant responses to glacial conditions by analyzing preserved wood from trees that actually lived during the last glacial period. The carbon isotope analysis provides some of the first direct evidence that glacial plants remained near their

lower carbon limit until the beginning of the glacial-interglacial transition, and also suggests that environmental factors that dominate carbon-uptake physiology vary across geologic time. The ring width analysis surprisingly shows that operating under limiting carbon conditions did not reduce growth in glacial trees. To state it another way, recent increases in [CO_2] have not produced a growth enhancement in modern trees, likely due to environmental constraints on growth, and adaptive and evolutionary constraints to low [CO_2] that are still present in long-lived plant species. While the carbon isotope and growth analyses focused on interannual physiological responses, the oxygen isotope analysis related more to broad-scale atmospheric circulation patterns. The results of this analysis indicate altered physiological strategies under less water-limited growing conditions, which impact the strength of plant responses to anomalous climatic events. Collectively, these results have serious implications for understanding of glacial plant function, estimating ecosystem-scale responses such primary productivity, and developing paleo-proxies for global atmospheric circulation patterns.